Fourth Symposium on

ORAL SENSATION AND PERCEPTION

Development in the Fetus and Infant

ORAL SENSATION
AND PERCEPTION

Sponsored by
THE NATIONAL INSTITUTE OF DENTAL RESEARCH
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Development in the Fetus and Infant

Edited by

JAMES F. BOSMA, M.D.

National Institute of Dental Research

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Development in the Fetus and Infant

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JAMES F. BOSMA, M.D.

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PREFACE

The Fourth Symposium on Oral Sensation and Perception reports an invitational conference held at the Stone House of the National Institutes of Health, November 20–22, 1972, sponsored jointly by the National Institute of Dental Research and the John E. Fogarty International Center for Advanced Study in the Health Sciences.

Because of the rapid accumulation of information and insights concerning antenatal and early postnatal structure and function in the oral and pharyngeal area, this Fourth Symposium, subtitled Development in the Fetus and Infant, overlaps the Third Symposium, subtitled The Mouth of the Infant.

This volume is based on papers presented at the conference and ensuing discussions.

The three previous volumes of this Symposium series were published by Charles C. Thomas Company in 1967, 1970, and 1972, respectively.

INTRODUCTION

MILO D. LEAVITT, JR.

Since so many of you attended the Symposium two years ago, I thought I would not review the history of the Fogarty Center as I had done on that occasion but rather use this occasion to greet you and perhaps bring you up to date on some of the things that we are doing in the Center.

You will recall that it was Mr. Fogarty's hope that this Center would concern itself with international collaboration and cooperation, that it would take a very close look at those aspects of science that bear on human society, and that it would act as a catalyst to undertake new things. What I thought I would do is tell you a few of the new things that are happening in the Fogarty Center and here on the campus that involve international activities.

One of the important events that occurred at NIH following the President's visit to Moscow this spring was the development of a US-USSR medical science program which is now well established, involving the Cancer Institute, the Heart and Lung Institute, and the National Institute of Environmental Health Sciences.

In this particular program, the Soviets fund their own research activities and we fund ours. We send scientists to the Soviet Union for varying periods, from a week or a month to, in one case, a year; they do the same. And in each case, the program is thoroughly outlined. The Heart Institute is very much interested in arteriosclerosis, the problems of arteriosclerotic heart disease, myocardial metabolism, and congenital heart disease. So their program for this next year is devoted to those four subjects.

The National Cancer Institute, on the other hand, has four areas of concern: cancer chemotherapy, the immunotherapy of human tumors, the viruses of leukemias and tumors in monkeys and humans, and the genetics of tumor cells. I could elaborate on each one of these but this will give you some idea of the things they are considering in this program.

Dr. David Rall at the National Institute of Environmental Health Sciences is very much involved in studies of methodology for the assessment of the biological effect of inhaled chemicals, those ingested, and those that are both inhaled and ingested. So much for that particular program.

The Fogarty Center is continuing its own studies of Soviet medicine and public health, and we have recently published a volume on nutrition in the Soviet Union. We produce these documents as background books for teams of scientists who are going to the Soviet Union on a particular mission.

We are also very much interested in Chinese medicine. A group of physicians from mainland China visited us this autumn and spent several days with us, and we expect that, when certain arrangements are made with mainland China, that a cooperative medical program will be set up. So we are preparing for that possibility.

We have had over the last three years a series of conferences dealing with human genetics and the problems that surround advances in human genetics such as the ethical, the legal and moral issues which may arise.

NIH has recently taken a very close look at human experimentation and the use of clinical trials. Because of the legal and ethical considerations involved in the use of human subjects, we are shortly embarking on a program of conferences and seminars to consider the variety of policy issues that relate to human experimentation.

The last thing I would like to mention is that we have initiated this past year a series of conferences on preventive medicine. Most of us recall preventive medicine as being less than an attractive specialty when we were students in medical school. However, everyone from the Chief Executive on down has been very much interested recently in what we can do to prevent certain diseases or conditions.

Since this renewed interest in prevention ranges throughout the Institutes and Divisions here at NIH, the Fogarty Center seemed to be the logical place to undertake such an activity. We have now set up committees to consider fetal development; communicable disease; trauma, emergencies, and emergency care; gastrointestinal diseases; derangements of dental health, under the direction of Doctor James Carlos of the Dental Institute; and panels on diabetes; obesity; bone diseases; toxemia of pregnancy and prematurity. These are all items believed to be of high priority. We would like to examine them carefully, produce documents that reflect the state of the art and which give us some idea where we are and what we can do in the way of prevention. We would like also to learn if certain preventive measures that are already available to us have failed and, if so, why they have failed. We would like to have some idea of the costs to undertake a full program of preventive medicine. We expect our first documents on this new program to be available sometime in the spring.

With that, I think I will close my comments, and again welcome you to this Fourth Symposium. It is a pleasure to have you here at the Fogarty International Center.

Thank you.

OPENING REMARKS

Richard C. Greulich

It is a considerable pleasure for me to welcome you on behalf of my colleagues at the National Institute of Dental Research, and on behalf of Dr. Seymour J. Kreshover, our Director, on the occasion of this Fourth Symposium on Oral Sensation and Perception. I have had the privilege of participating in the planning of these four Symposia, and am proud to have had a role in fostering their growth. They have matured in terms of the increasing number of professional fields represented, and also in terms of the scope, quality and significance of their content. The program for this Fourth Symposium suggests that further progress will be forthcoming in the next three days.

This subject area of oral sensation and perception is not a major focus of interest of the National Institute of Dental Research. Our resources in dollars and in manpower are committed to systematic scrutiny of the causes and amelioration of the major diseases of the oral cavity. But, I recognize that this aspect of oral function is a new and important subfield of research endeavor, and this Institute welcomes this opportunity to sponsor it.

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ORAL SENSATION AND PERCEPTION

Chapter 1

PROLOGUE TO THE SYMPOSIUM

James F. Bosma, M.D.

This symposium is assembled in a context of rapidly accumulating information and understanding about the structure and the sensorimotor functions of the infant's mouth and pharynx. According to the previous symposia of this series, descriptions of the neuroembryology of this region and of its developing exteroceptive sensorium are progressing very well. We have been given a good start on neuroanatomical description of the central arrangement and interconnections of the branchial nerve nuclei in the fetus. The gross anatomy of this region in the term fetus has been described.

The complexly organized performances of sucking and of suckle feeding continue to be profitably studied. We increasingly appreciate the remarkable adaptations of the newborn to the circumstances of feeding. Moreover, we are also becoming more cognizant of the role of sucking as an expression of the response state of the infant and as a reflection of the infant's discrimination in other sensory systems, such as vision and audition. Description of the gestures and sounds of infant crying is continuing. And additional simple oral reflexes are being discovered.

It is apparent that research on anatomical and physiological development of the mouth and pharynx can continue in a discovery motif for some time. The exteroceptors of this area are heterogeneous, and their heterogeneity is compounded by variations in the schedule of their development. Description of these schedules is needed as a basis for further studies of local sensorimotor performances. We are also awaiting developmental description of the proprioceptors in the branchially-derived musculature. And we are in need of more comprehensive descriptions of the histologic development of the brain stem in the fetus and the infant.

Understanding of the sensorimotor functions of the mouth and pharynx is correspondingly incomplete. We have not yet compiled the full list of functions, or even of the categories of functions, which the infant pharynx and mouth can accomplish. This is probably because we have not had the insight or ingenuity to look for performances other than those which we have so far recognized. The key to further description of these functions lies in the study of exteroception. In view of the changes in mucosal receptors through time, such inquiries should be developmentally dated in relation to neurohistology. Their reference should also be physiological, in comparison with development in the currently recognized position, respiratory and feeding functions of this region.

But our concern beyond the local functions is that of their influences upon the developing brain. These influences cannot yet be estimated. Somewhat empirically, we describe neurological development as a resultant of the interaction of basic patterns in the CNS with a substrate or matrix of sensory experience, principally from sensorimotor performances. A primary variant in this factor of experience is the individuality of the sensorium. These individual variations are compounded by the heterogeneity of life experience. Both of these mechanisms of variation are modified by peripheral somatic anomalies, such as cleft lip and palate or hypoplasia of the tongue. Both of these mechanisms of variation are modified by peripheral neurological anomalies, such as congenital sensory neuropathy or familial dysautonomia. We should expect that the anomalies of exteroception are associated with other neurological abnormalities. Thus, aplasia of taste buds is but a part of the various neuropathologies which comprise the syndrome of familial dysautonomia.

We encourage one another toward further specifications among this heterogeneity of sensory and central neurological structures and functions. But we must urgently join in efforts to resolve and relate this increasing diversity which we are finding. Our obligation is toward an integrative approach. For, unless the infant is unexpectedly different from the mature organism, he continually resolves the potential heterogeneity of his oral sensory inputs into sequential composite entities of action and experiences. His resolutions pertain to the current moment; his orientation to life is existential.

The young infant's mouth and pharynx—his "portal area"—is a major portion of his experiential world. His afferent information is probably received from this area in the form of synergies of particular experience, whether that experience has generated a response or has been generated by it. One of our target questions, then, should be the specification of the sensory synergies which are developmentally inscribed upon the maturing brain.

Another area for exploration is the interaction of portal area experience with development of pertinent parts of the central nervous system. This interaction is continuous, beginning at the time in development when this peripheral area is capable of generating sensory experience. In fetal life, when nutrition and respiration are accomplished at the placenta, the mouth and pharynx are negotiating with the amniotic environment, partitioning it into physiological units and manipulating these units in rehearsal of swallow, suck and breath. These three master coordinations are matured as entities and in schedule relation to each other.[12] Because of this, the infant can so readily adapt to the different fluid—air—on the occasion of birth. In this perspective of the portal area, birth is an incident. It can effect no change in peripheral receptors, though the shift in media changes the information they receive. This change is quantitative, rather than qualitative. Birth can effect no change in central representation of portal area functions, except as these may be modified by respiratory deficiency or metabolic disturbances. Correspondingly, the motor gestures of environment manipulation should vary only in dimension, with the same basic synergies in operation which had been employed in the fetus.

In postnatal development, the portal area of the human changes greatly. It acquires a variety of oral feeding maneuvers, appropriate to an omnivore. In distinction from other mammals, the human acquires an oral chamber. And the pharynx enlarges, principally by a species distinctive elongation of the pharynx in relation to the cervical vertebrae and to the facial skeleton. This developmentally expanded pharynx and mouth are the resonance chamber of human speech. The human muscular palate, tongue and lips which articulate speech are not remarkably different in structure and form from those of other mammals. Their postnatal anatomical adaptations are chiefly those which make them capable of mobility in relation to the expanded mouth and pharynx. In the postnatal development of this area, man becomes distinguished among the mammals by his potential for egressive manipulations of respiration, more than his capacity for ingessive manipulation in feeding. The coordinations of the palate, tongue and lips in speech articulation are the ultimate performance elegancy of the portal area. Man is the speaking mammal.

In the perspectives of this symposium, the acquisition of the capacity for expressive speech is the result of related maturations in sensory input and in anatomical form of the performing area and its central representation. This is supplemented, of course, by the precedents and examples of expressive communications which impinge upon the infant and child from his social environment. We should anticipate the discovery of a rich oral sensory resource in man, corresponding to the richness of man's achieved central representations of functions.

Whatever its generative mechanisms, the sensorimotor achievements of the portal area are autogenous. The infant organizes and governs its sensorimotor world of experience in relation to its own current needs and purposes. In doing so, the infant is generating his own future.

When we sufficiently understand the functions of the portal area in the human infant, we should be able to recognize the antecedents of the performance changes in this area which distinguish the child from the infant. The relationships of these neurological generations to the developmental changes in spatial relations in the mouth and pharynx are hypothetical. Hypothetical, also, is the influence of these changes in dimension and contour of the pharyngeal and oral chambers upon the development of articulate speech. Clearly, the sensory inputs from the portal area are modified in correlation with these gross anatomical changes.

Sorting out the primary and derivative mechanisms in these postnatal changes in peripheral anatomy and in centrally represented functions will be facilitated by clinical studies of the syndromes of neurologic impairment in which these maturations are delayed or do not occur. Conclusive description of these related generations of human function and form of the portal area, however, must be gained through study of normal subjects.

Most of this symposium is concerned with infants who are without evident neurologic or somatic impairment. The clinical variation which we will consider is that of prematurely born infants. In contrast with the actively performing mouth of the termborn infant, the mouth of the premature is commonly open at the lips and the anterior part of tongue. It is our clinical impression that the mouth of the premature is inactive in comparison with its capabilities. This impression is supported by an interesting study of Dubignon, Campbell and Partington [7] of non-nutrient sucking by prematures on a recording nipple during a two minute period. They found that sucking at mature *rate* was achieved at the 34th postconception week. But the *number* of sucks in the test period did not reach maturity until the 37th week.

Pharyngeal and laryngeal functions of the premature are apparently more ready, more competent, than oral functions. For example, positional function of the pharynx—its use as an airway—is present in the viable premature. Swallowing, developmentally the most primitive function, is present from the fifth month of gestation.[8] And the cry of the small premature is well-organized in its tonal composition.[15]

We should be concerned about possible deficiencies of oral experience in premature infants who traverse our modern nursery routines. These concerns are but a regional focus of the more general concerns of clinicians about the premature's loss of living experience. This matter of deficiency in living experience, and its possible relation to later-demonstrated developmental deficiencies, has been comprehensively reviewed by Wright[16] and others.[1,2,3,4,7,9,10,11,14]

Attempts have been made on programmatic or more casual routines to compensate for this deficiency by rocking or carrying, and by auditory or visual stimulation.[16] Tactile and kinesthetic experience have been shown to be particularly effective in facilitation of visual and manual performance.[16]* But there are no reports of attempts to increase the oral experience of prematures. It is appropriate to consider routines of oral positioning and stimulation designed in analogy to normal functions of the mouth.

A simple and obvious compensation for apparent deficiencies in oral experience would be to insure sufficient apposition of oral structures, and sufficient fluid within the appositions. The choice of a saliva supplement or surrogate deserves special consideration, apropos of the potency of chemosensation. A prosthesis, adapted from a standard infant pacifier, could be employed to enhance oral apposition.

But the difference in oral sensory experience of the infant during premature care is more than deprivation of primary sensation. The central sensory connections must also be immature, and unready for the sensations which are imposed upon them. There must, therefore, be a qualitative difference in the sensory experience during premature life. This difference in experience may be the background of later-manifested aberrations of responses to sensory stimuli which have been noted by Mary Shirley.[13,14] She observed auditory and visual hyperacuity in these infants and children. And suggested that the child who had been premature "is highly receptive to stimulation, particularly to auditory stimulation; but he is less capable than the term child of making an adequate motor adjustment to his stimuli". This residual aberration of sensation control following prematurity is appropriate to the current concepts of neurological maturation as a process of

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*Scarr-Salapatek and Williams report on "The Effects of Early Stimulation on Low-Birth Weight Infants" in *Child Development* 44:94–101 (March, 1973). An experimental group of 15 prematures, matched with controls, were given supplemental visual, tactual and kinesthetic stimulation in the nursery and subsequently by mothers at home. After 4 weeks in the nursery, the experimental prematures showed slight-to-significant advantages on the Brazelton scales, compared with controls. At one year, the experimental infants demonstrated an average of nearly 10 IQ points above controls.

The authors make the significant point that "different forms of stimulation may be necessary for extrauterine development than intrauterine maturation regardless of gestational age". (p. 95).

selective diminution of sensory inputs by acquisition of inhibitory control. In application by analogy, the premature infant's oral sensorium may be an insufficiently guarded gate, affording an excessive and inordinate access of experience.

In light of these general concerns and precautions, experimental provision of oral experience is necessarily empirical, in our current limitation of understanding. We know little about the normal or appropriate oral experience of the fetus during its premature life. Certainly we do not know the mechanisms or processes whereby this experience is incorporated into the matrix of postnatal development. On the basis of analogy from development in other systems, we can only suggest that appropriate oral experiences may be critical in the final weeks of gestation, and that their interruption may impair fragile syntheses of central representations of functions.

Such experimentation should be oriented to available information about the sensory capabilities of the mouth. Perhaps these infants who are found to be hypersensitive to sounds, subsequent to their premature care, are also excessively sensitive to oral mucosal stimulation. There is a very real possibility that oral experiences selectively imposed on the premature's developmental calendar is excessively potent and could result in profitless or damaging habits.

Evaluation of the effects of supplementary oral experience would depend upon our perspectives of the role of such experience in development. The most naive evaluations would be of oral functions in feeding: the graduation into biting and chewing and into mature pattern of swallow. Oral gestures in play might provide useful criteria.[16] The more elegant development of expressive speech would, most likely, be more liable to disruption. Speech skills in children who had been premature have been found to be delayed [3] or impaired.[2,11] Drillien,[5,6] in a broad scale neurological assessment of children who had been born prematurely, found that speech development was the most common area of impairment.

The patterns of utilization of the mouth as an affective resource may also be of particular significance. We might inquire, also, about the general incidence and occasion of rumination and of tongue sucking in young children who had been prematurely born and had experienced weeks or months of incubator care. Variations in preferences of taste, in volume of ingestion and in affective parameters of intake could also be evaluated.

The portal area of the infant, while engaged in the manipulation of air and of the nipple and milk, is also a primary site of empathic linkage between the infant and mother. This linkage is implemented at suckle feeding, at nonnutritive sucking and in other interpersonal manipulations of the mouth and head, and at the social occasion of cry and other responsive sounds. The premature infant in the incubator lacks each of these mechanisms of linkage. Although we can hardly expect to accomplish extra-uterine substitution for the experience of the fetus as a continuous receiver of experience from the mother's internal world, it might be possible to increase the maternal parameters of care in the premature's world.

mature *rate* was achieved at the 34th postconception week. But the *number* of sucks in the test period did not reach maturity until the 37th week.

Pharyngeal and laryngeal functions of the premature are apparently more ready, more competent, than oral functions. For example, positional function of the pharynx—its use as an airway—is present in the viable premature. Swallowing, developmentally the most primitive function, is present from the fifth month of gestation.[8] And the cry of the small premature is well-organized in its tonal composition.[15]

We should be concerned about possible deficiencies of oral experience in premature infants who traverse our modern nursery routines. These concerns are but a regional focus of the more general concerns of clinicians about the premature's loss of living experience. This matter of deficiency in living experience, and its possible relation to later-demonstrated developmental deficiencies, has been comprehensively reviewed by Wright[16] and others.[1,2,3,4,7,9,10,11,14]

Attempts have been made on programmatic or more casual routines to compensate for this deficiency by rocking or carrying, and by auditory or visual stimulation.[16] Tactile and kinesthetic experience have been shown to be particularly effective in facilitation of visual and manual performance.[16]* But there are no reports of attempts to increase the oral experience of prematures. It is appropriate to consider routines of oral positioning and stimulation designed in analogy to normal functions of the mouth.

A simple and obvious compensation for apparent deficiencies in oral experience would be to insure sufficient apposition of oral structures, and sufficient fluid within the appositions. The choice of a saliva supplement or surrogate deserves special consideration, apropos of the potency of chemosensation. A prosthesis, adapted from a standard infant pacifier, could be employed to enhance oral apposition.

But the difference in oral sensory experience of the infant during premature care is more than deprivation of primary sensation. The central sensory connections must also be immature, and unready for the sensations which are imposed upon them. There must, therefore, be a qualitative difference in the sensory experience during premature life. This difference in experience may be the background of later-manifested aberrations of responses to sensory stimuli which have been noted by Mary Shirley.[13,14] She observed auditory and visual hyperacuity in these infants and children. And suggested that the child who had been premature "is highly receptive to stimulation, particularly to auditory stimulation; but he is less capable than the term child of making an adequate motor adjustment to his stimuli". This residual aberration of sensation control following prematurity is appropriate to the current concepts of neurological maturation as a process of

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*Scarr-Salapatek and Williams report on "The Effects of Early Stimulation on Low-Birth Weight Infants" in *Child Development* 44:94–101 (March, 1973). An experimental group of 15 prematures, matched with controls, were given supplemental visual, tactual and kinesthetic stimulation in the nursery and subsequently by mothers at home. After 4 weeks in the nursery, the experimental prematures showed slight-to-significant advantages on the Brazelton scales, compared with controls. At one year, the experimental infants demonstrated an average of nearly 10 IQ points above controls.

The authors make the significant point that "different forms of stimulation may be necessary for extrauterine development than intrauterine maturation regardless of gestational age". (p. 95).

selective diminution of sensory inputs by acquisition of inhibitory control. In application by analogy, the premature infant's oral sensorium may be an insufficiently guarded gate, affording an excessive and inordinate access of experience.

In light of these general concerns and precautions, experimental provision of oral experience is necessarily empirical, in our current limitation of understanding. We know little about the normal or appropriate oral experience of the fetus during its premature life. Certainly we do not know the mechanisms or processes whereby this experience is incorporated into the matrix of postnatal development. On the basis of analogy from development in other systems, we can only suggest that appropriate oral experiences may be critical in the final weeks of gestation, and that their interruption may impair fragile syntheses of central representations of functions.

Such experimentation should be oriented to available information about the sensory capabilities of the mouth. Perhaps these infants who are found to be hypersensitive to sounds, subsequent to their premature care, are also excessively sensitive to oral mucosal stimulation. There is a very real possibility that oral experiences selectively imposed on the premature's developmental calendar is excessively potent and could result in profitless or damaging habits.

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Chapter 2

THE MIGRATORY BEHAVIOR OF NEURAL CREST CELLS

Drew M. Noden

I. INTRODUCTION

It is most appropriate to begin a symposium on the functional and anatomical aspects of oral development with a consideration of the ontogeny of cells derived from the embryonic neural crest. These cells play an essential role in the formation of many components in the vertebrate head, including structural elements and peripheral neurons.

The cells derived from this population are unique among vertebrate embryonic systems in exhibiting the following three characteristics:

1. Individual neural crest cells possess the capacity to migrate away from their origin and move in a highly patterned manner into specific regions of the embryo;
2. The neural crest population gives rise to a great variety of cell types (Tables 2–1 and 2–2), most of which are biochemically and cytologically dissimilar;
3. There exists a strict correlation between the final position and relationships (dispersed, aggregated, within an epithelium, etc.) of neural crest cells and the nature of their terminal cytodifferentiation. In the acquisition and expression of these three characteristics neural crest cells touch upon many fundamental problems in developmental biology.

Following brief reviews of neural crest cell origins and derivatives, this Chapter will focus on their migratory behavior, considering the normal patterns of neural crest migration in the head regions of the chick embryo (section IV) and an experimental analysis of the genesis of these patterns (section V). The Chapter concludes with a discussion of intra-embryonic environmental influences which might be affecting neural crest cell migratory behavior and cytodifferentiation.

II. FORMATION OF THE NEURAL CREST

The neural crest population forms in a rostro-caudal manner from the dorsal margin of the neural tube during or immediately following neurulation (neural tube closure). Two stages in the formation of the neural crest in the mesencephalic region of the chick embryo are illustrated in Figures 2–1 and 2–2. These

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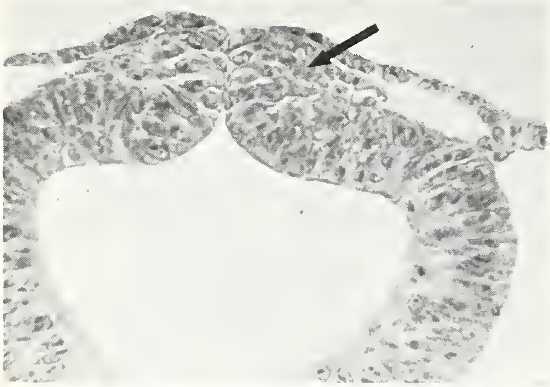

Figure 2–1. Chick mesencephalon, stage 9, showing the apposition of the neural folds and onset of neural crest (arrow) formation.

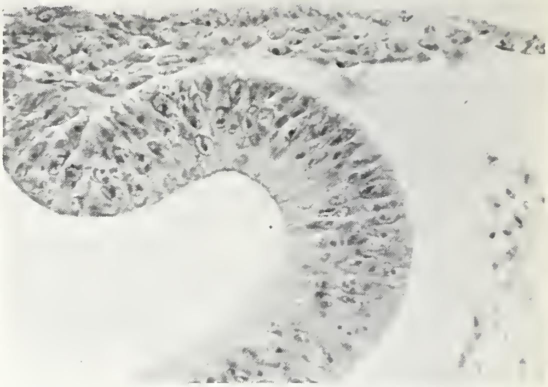

Figure 2–2. Chick mesencephalon, stage 9½, at onset of neural crest emigration.

cells are rapidly dividing and continue to do so during their subsequent migrations.

The motive force which causes a newly formed neural crest cell to emigrate from its site of origin is unknown. Hypotheses such as negative chemotaxis,[42,43] release from contact inhibition,[45] migration along an increasing adhesive gradient,[1] or a switch in the relative strengths of intercellular adhesion from isotypic (crest:crest interactions) to heterotypic (crest:non-crest cell interactions) [49,52] have been proposed, but the molecular basis for the change in behavior from a non-motile to a motile condition has not been elucidated.

Accurate cell counts of the neural crest population have not been made. However, my own observations on the chick show that there are many more crest cells per unit of axial length in the cephalic regions than in the trunk. Moreover, emigration of the entire neural crest population from a specific cephalic region requires only four to six hours, while in a region of equivalent size in the trunk this process would last at least three times that long.[46] Additional regional differences exist with respect to the fates of head and trunk neural crest cells, as summarized in Tables 2–1 and 2–2. These quantitative and qualitative differences have to be taken into consideration in analyzing the data from heterotopic neural crest transplantation experiments to be discussed later (section V).

III. DERIVATIVES OF THE NEURAL CREST

The neural crest origin of spinal and, in part, cranial sensory ganglia, autonomic neurons, pigment cells (except those of the retina), chromaffin cells, odontoblasts, some glial and Schwann sheath cells, some cephalic dermal bones, and visceral arch cartilages has been well documented; [17,47,48] (refer to Tables 2–1 and 2–2). Recent evidence, which will be discussed in Chapter 3, indicates that several types of secretory cells might also be of neural crest origin.[23,27]

This diversity of cell phenotypes presents a tremendous challenge to the developmental biologist: How do so many

TABLE 2–1

NEURAL DERIVATIVES OF THE NEURAL CREST

Tissue or Structure	*Cranial Crest Derivatives*	*Trunk Crest Derivatives*
1. Sensory Ganglia	trigeminal (V) geniculate (VII superior (root IX) jugular (root X)	spinal ganglia
2. Parasympathetic (cholinergic) Ganglia	ciliary ethmoid sphenopalatine submandibular enteric system	pelvic plexus Remak's ganglion enteric system
3. Sympathetic (adrenergic) Ganglia		superior cervical prevertebral paravertebral (chain)
4. Accessory Cells	glia cells Schwann sheath cells some meningeal cells	

TABLE 2–2

NON-NEURAL DERIVATIVES OF THE NEURAL CREST

1. Pigment Cells	melanophores xanthophores iridophores (Naevus cells)
2. Skeletal and Connective Tissues (derived from head crest only)	membrane bones (osteoblasts) visceral cartilages (chondroblasts) anterior trabecular cartilage part of the basal plate some parachordal cartilage odontoblasts sclera and choroid optic coats
3. Trunk Connective Tissues (anamniotes)	mesenchyme of dorsal fin
4. Secretory Cells	calcitonin—secreting "C" cells ACTH—secreting corticotroph cells MSH—secreting cells adrenal medullary chromaffin cells carotid body Type I cells

different cell types derive from an (apparently) homogeneous initial population? It has been shown that cytodifferentiation of some cell types, for example visceral arch cartilages,[8,16,26] sympathoblasts,[4] and odontoblasts [22,30] is dependent upon inductive tissue interactions. However, the factors responsible for the determination of the other neural crest cell types have not been clearly defined.

Evidence from *in vitro* studies indicates that there is a correlation between the spatial arrangement of some neural crest cells and their eventual cytodifferentiation. Dispersed cells tend towards the melanogenic pathway,[5,42] while tightly clustered crest cells undergo neurogenesis.[49] Whether this correlation is coincidental or causal remains to be proved.

These data do indicate that a more thorough analysis of the genesis of neural crest migratory patterns must be undertaken. Such an investigation might help elucidate the factors directing neural crest cytodifferentiation. The remainder of the Chapter focuses on an analysis of the migratory behavior of cephalic neural crest cells in the chick embryo.

IV. MIGRATORY BEHAVIOR OF CEPHALIC NEURAL CREST CELLS

Migratory behavior refers to the complete set of movements and interactions by which individual neural crest cells migrate to and become localized at specific regions of the embryo. It includes the highly organized associations which crest cells form with other cells of both crest and non-crest origin during their migration and at their terminal locations. The patterns of trunk crest cell migration have been described by Detwiler [6] and Weston [46] for urodele and chick enbryos, respectively. The more complex migratory patterns found in the head regions have been experimentally revealed in amphibians by Hörstadius and Sellman [18] and Chibon,[3] and in the chick by Johnston [18] and Noden.[25]

Neural crest cells possess no definitive marker by which they can be distinguished from mesodermal or placode-derived cells. In order to follow the normal migratory behavior of these cytologically indistinct cells through the pre-otic regions of the chick embryo, neural fold tissue was transplanted orthotopically from a ^{3}H-thymidine labeled 32-hour donor into an unlabeled host embryo of the same age, as illustrated in Figure 2–3. The hosts were sacrificed between 36 and 96 hours of total incubation and staged according to the Hamburger-Hamilton stage series.[11] The position of all labeled cells was ascertained radioautographically, and reconstructions were made of representative cases.

The pre-otic region of the chick embryo has been divided into five areas (Figure 2–3) which correspond approximately to the morphological subdivisions of the embryonic brain listed in Table 2–3. The migratory behavior of crest cells in Areas 4 and 5, Area 3, and Area 2 will be described separately as the patterns in each of these areas are unique. Very few, if any, crest cells are formed in Area 1.

1. Migratory Patterns, Areas 4 and 5

These two rhombencephalic areas show similar patterns of neural crest migration, and the following account of

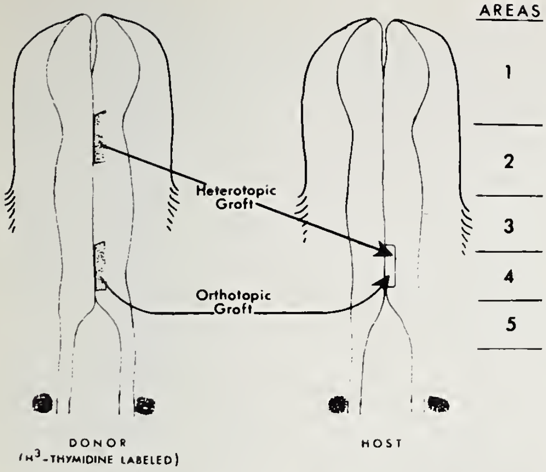

Figure 2–3. Scheme of neural crest transplantations. Note the approximate delineation of Areas (see text and Table 2–3).

Area 4 crest cell movements applies as well to Area 5. As the emigrating population of crest cells leaves its dorsal origin, its members completely fill the space between the metencephalon and the lateral body epidermis (Figure 2–4). While many of these cells then continue migrating towards the lateral margin of the pharynx, some neural crest cells cease their ventral migration and aggregate beside the metencephalon (Figures 2–5 and 2–6). This condensation, detectable at stage 12 (approximately 12 hours after transplantation), marks the beginning of formation of the trigeminal ganglion. Growth of the ganglion proceeds by recruitment of nearby crest cells, proliferation, and later condensation of cells derived from the mandibular and ophthalmic epidermal placodes.[10,20] Figures 2–7a, 2–7c, and 2–8 show the fifth and seventh (Area 5) cranial ganglia at two days of incubation (stage 14–15).

Most of the remaining Area 4 neural crest cells move around the anterolateral margin of the first pharyngeal pouch into the presumptive first (mandibular) visceral arch (Figures 2–5 through 2–8). After the crest cells migrate around and under the pharyngeal pouches they move caudally, as seen in the reconstructions (Figures 2–6 and 2–8). While a few of these cells become associated with the pharyngeal endoderm and use it as a substratum for further migration, most of the cells form a mesenchyme within the visceral arch. This mesenchyme is interspersed with vascular and embryonic connective tissues of meso-

TABLE 2–3

THE PRE-OTIC NEURAL CREST AREAS: LOCATION AND MAJOR DERIVATIVES

Area	*Corresponding Part of the Brain*	*Major Neural Crest Derivatives*
1	anterior prosencephalon	no neural crest cells formed
2	posterior prosencephalon anterior mesencephalon	upper facial and some maxillary region connective tissues; outer eye coats, corneal mesenchyme
3	posterior mesencephalon	maxillary process; ciliary ganglion; glial and Schwann sheath cells
4	metencephalon	mandibular cartilages; trigeminal ganglion and accessory cells
5	anterior myelencephalon (pre-otic)	hyoid arch cartilages; geniculate ganglion and accessory cells

dermal origin. However, by stages 16–17 the vascular network begins to degenerate, and mesodermal tissues condense into presumptive muscle plates[21]. A few crest cells become intimately associated with the endothelial wall of the ventral aorta and aortic arches; it has been suggested that these may be the primordia of pericytes (Johnston, personal communication).

The migratory pattern of Area 5 crest cells, which contribute to the formation of the geniculate ganglion (Figure 2–7c) and hyoid visceral arch (Figure 2–7d), is essentially the same. Figure 2–9 summarizes the early migratory pattern of Area 4 neural crest cells.

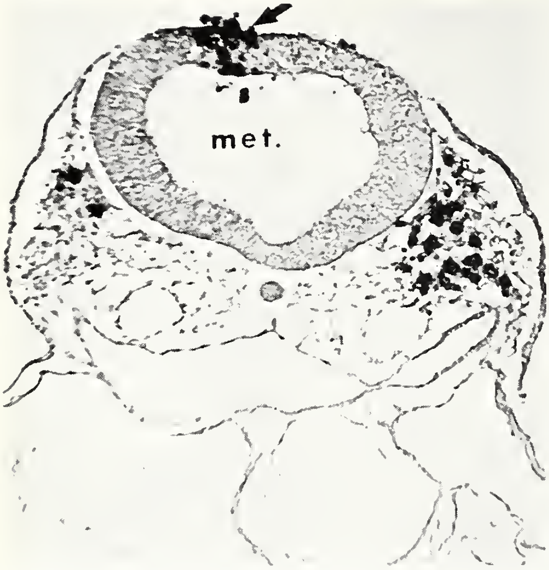

Figure 2–4. Radioautograph, Area 4, stage 12. Note spreading of labeled cells ventro-lateral to the metencephalon (*met.*). Arrow indicates the position of those grafted neural fold cells which healed in place, contributing to the roof of the metencephalon.

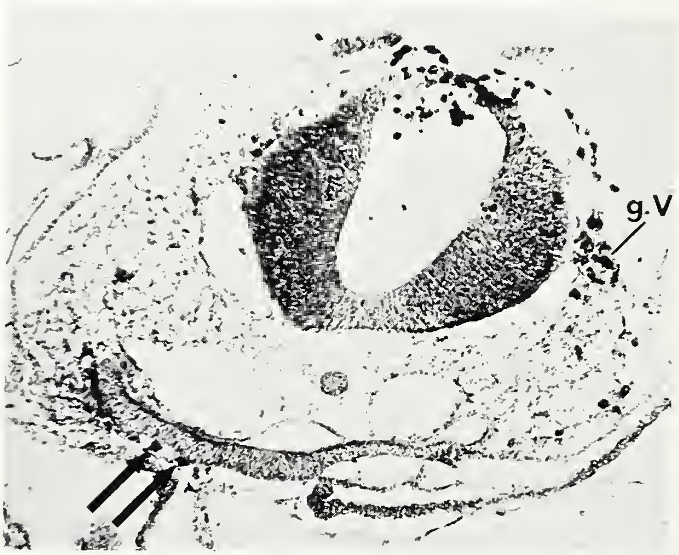

Figure 2–5. Radioautograph, Area 4, stage 13. Trigeminal ganglion (*g. V*) is well defined and heavily labeled. Arrows indicate labeled neural crest cells below the pharyngeal endodern. (cross section 146 on reconstruction Figure 2–6)

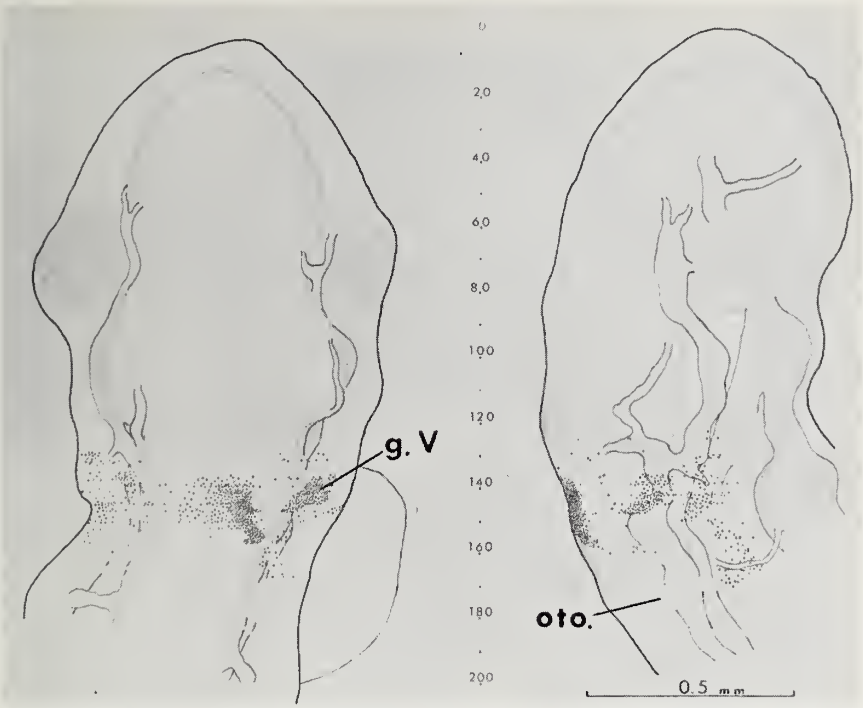

Figure 2–6. Reconstruction, stage 13 (left, dorsal view; right, lateral view), showing position of labeled neural crest cells following orthotopic Area 4 transplantation. Note the density of crest cells in the trigeminal ganglion (*g. V*) and their distribution below the pharynx. *Oto.*, otocyst. The vertical column of numbers indicates the position of the corresponding transverse section.

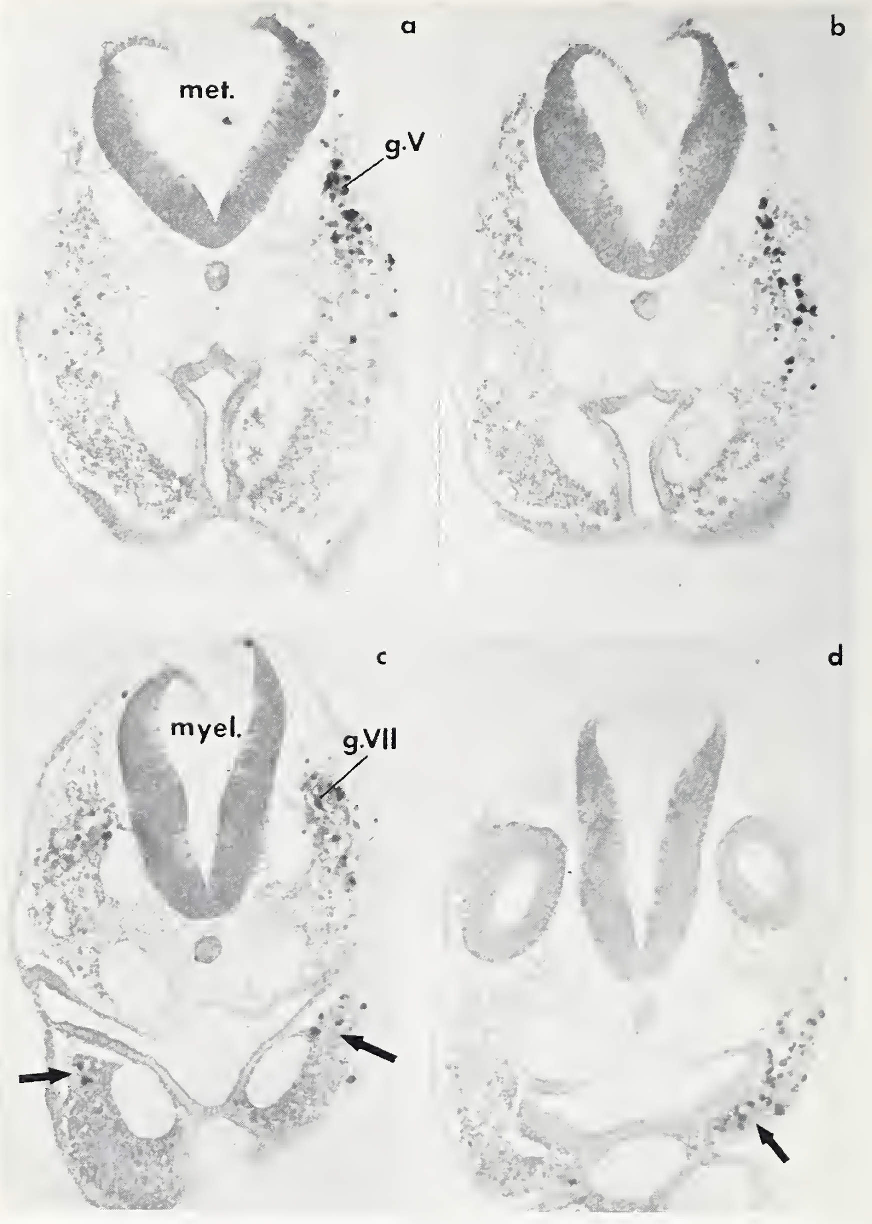

Figure 2–7. Radioautographs, Areas 4 and 5, stage 14$^+$. Transverse sections of embryo reconstructed in Figure 2–8. Note labeled cells in the trigeminal (*g. V*) and geniculate (*G. VII*) ganglia, the mandibular arch (arrows, section c), and the hyoid arch (arrow, section d).

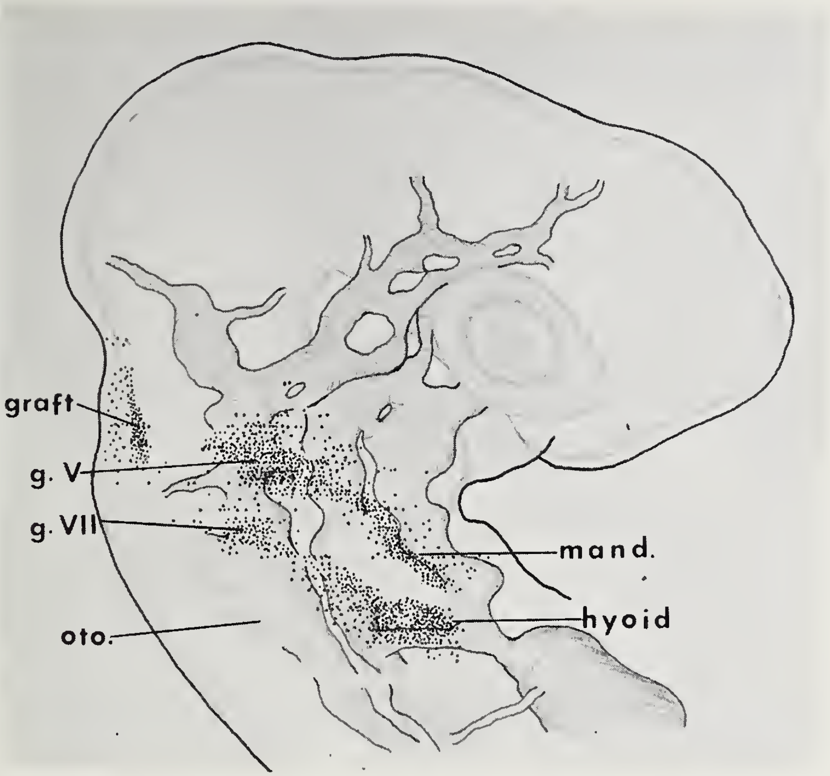

Figure 2–8. Reconstruction, stage 14^{+}. Note distribution of Area 4 crest cells in the trigeminal ganglion (*g. V*) and first visceral (*mandibular*) arch, and parallel positions of Area 5 crest cells in the geniculate (*g. VII*) ganglion, immediately rostral to the otocyst (*oto.*), and second (*hyoid*) visceral arch.

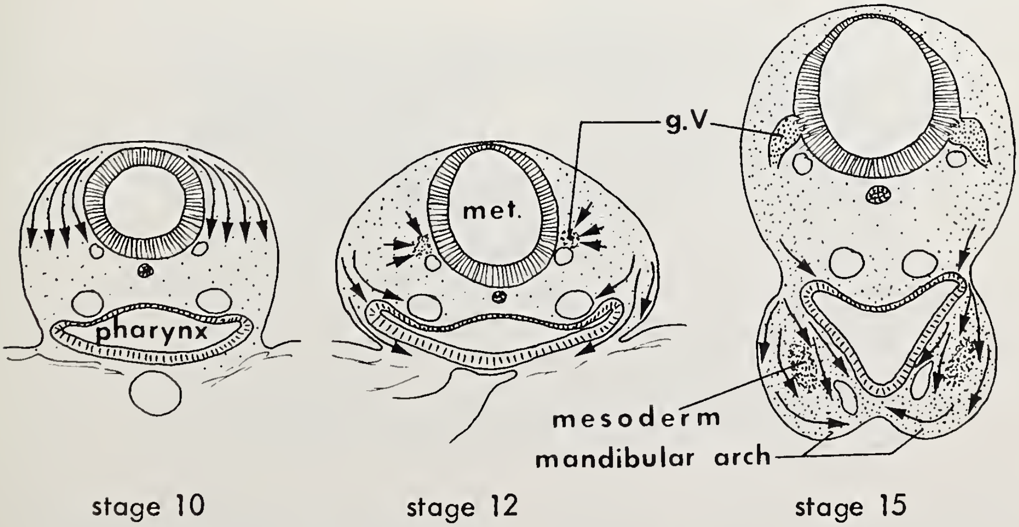

Figure 2–9. Summary of Area 4 early migratory patterns.

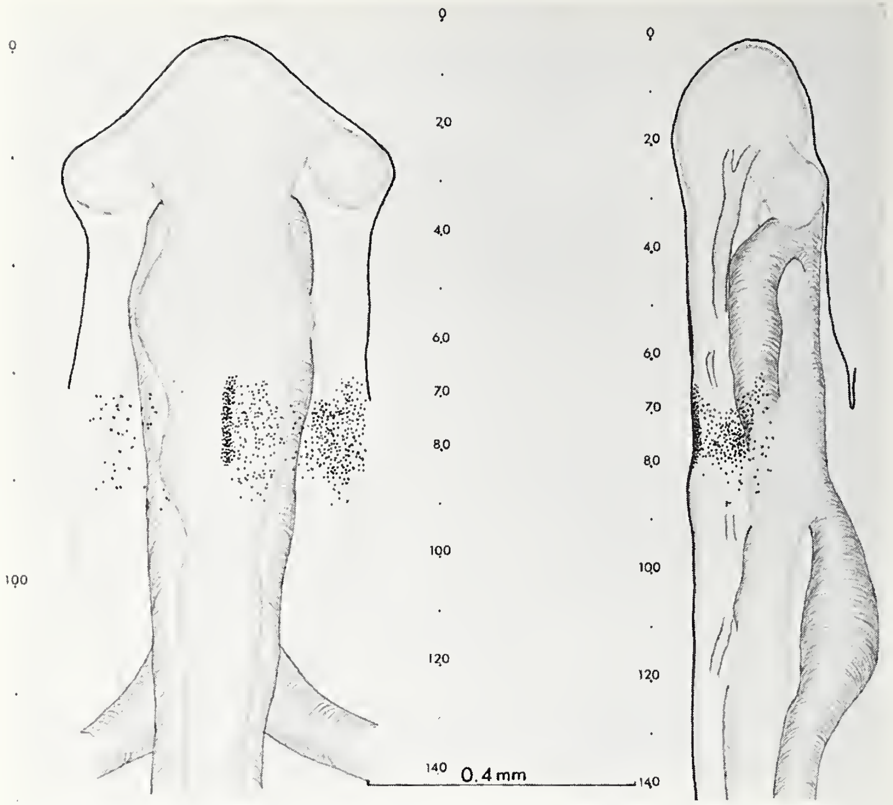

Figure 2–10. Reconstruction, stage 12 showing position of neural crest cells following an orthotopic Area 3 transplantation.

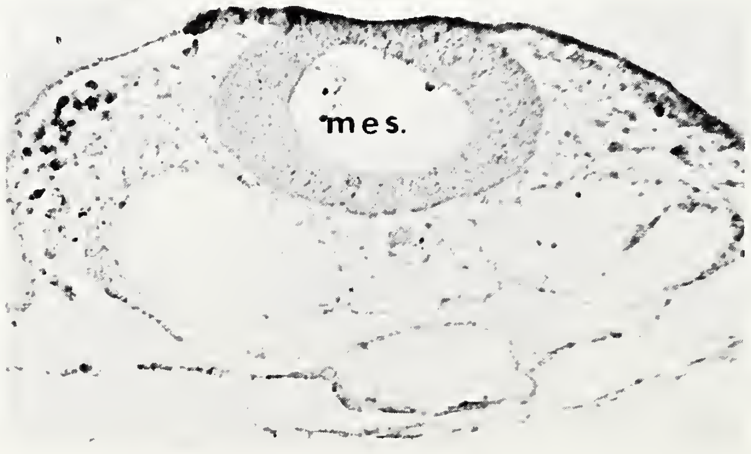

Figure 2–11. Radioautograph, Area 3, stage 12. Section 76 through reconstruction Figure 2–10. Note the *en masse* movement of cells between the epidermis and the mesoderm. No neural crest cells in this area migrate along the outer wall of the brain.

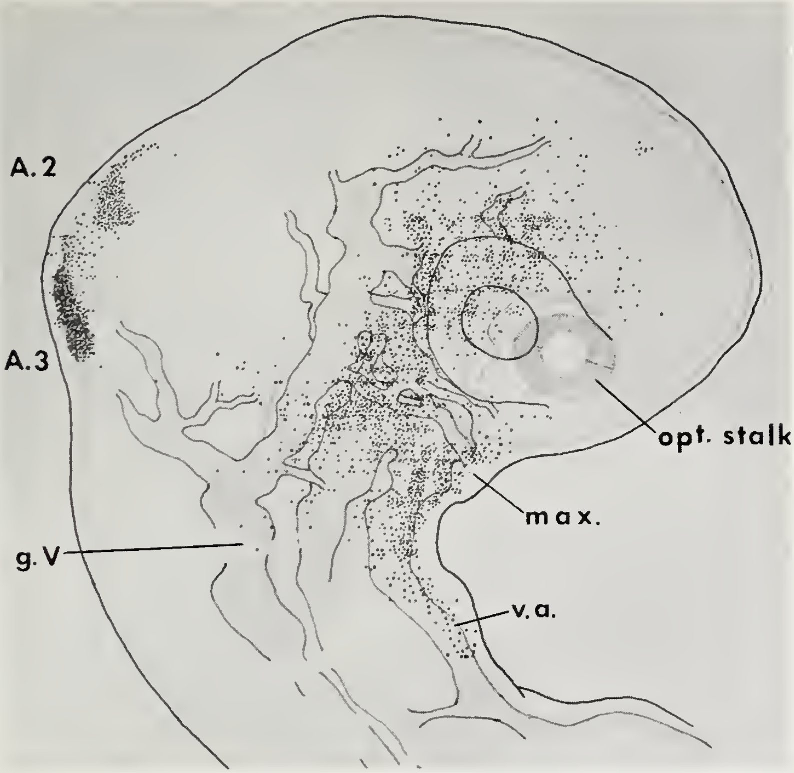

Figure 2–12. Reconstruction, stage 14. The implant split into two fragments which healed into Area 2 (*A. 2*) and Area 3 (*A. 3*). Labeled cells from A. 2 are located around and deep to the optic cup (shown in outline) and into the maxillary process (*max.*), along with A. 3 cells. Note the caudal position of some A. 3 cells below the pharynx, lateral to the ventral aorta (*v. a.*).

2. Migratory Patterns, Area 3

Area 3 neural crest cells emigrating from the posterior portion of the mesencephalon (Figure 2–10) display a pattern different from Area 4 crest cells in that the population remains compact beneath the surface of the epidermis and superficial to the mesodermal mesenchyme (compare Figure 2–11 with 2–4). These crest cells do not use the mesencephalon as a substratum and do not aggregate near the brain.

Most of the population moves en masse into the cranial part of the first visceral arch (Figure 2–12), with some cells migrating caudally beneath the pharynx to the level at which the second aortic arch leaves the ventral aorta. To reach this level by stage 14 the neural crest cells must be moving at approximately 1 mm per day.

During the third day of incubation some Area 3 crest cells invade the population of presumptive neuroblasts derived from the ophthalmic placode, and this mixed population joins the mandibulo-maxillary condensation to form the trigeminal ganglion. An aggregate of Area 3 crest cells forms behind the optic cup near the convergence of the ophthalmic and oculomotor nerves (Figure 2–13); this is the primordium of the ciliary ganglion. Figure 2–13 also reveals the presence of labeled presumptive Schwann sheath cells along the entire length of the oculomotor nerve. These crest cells must have become associated with the nerve when its fibers reached the area beneath the eye. Some of the cells then moved proximally along the nerve to its root. Whether there exist any factors which emanate from the growing nerve fibers and attract neural crest cells is not known. However, once the crest cells become associated with a nerve tract they use it as a substratum for directed migration. Figure 2–14 summarizes early Area 3 neural crest migratory patterns.

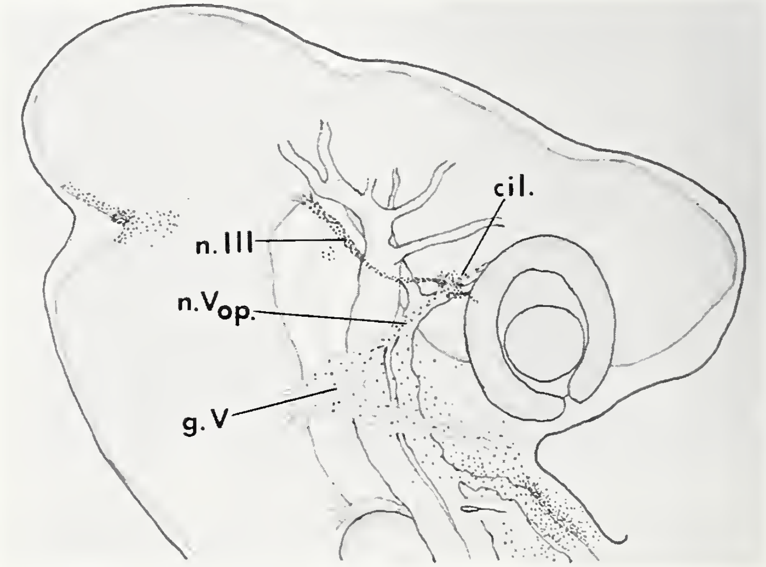

Figure 2–13. Reconstruction stage 16. Labeled Area 3 cells are found along cranial nerves III *(n. III)* and ophthalmic V *(n. $V_{op.}$)*, and are condensed in the presumptive ciliary ganglion *(cil.)*.

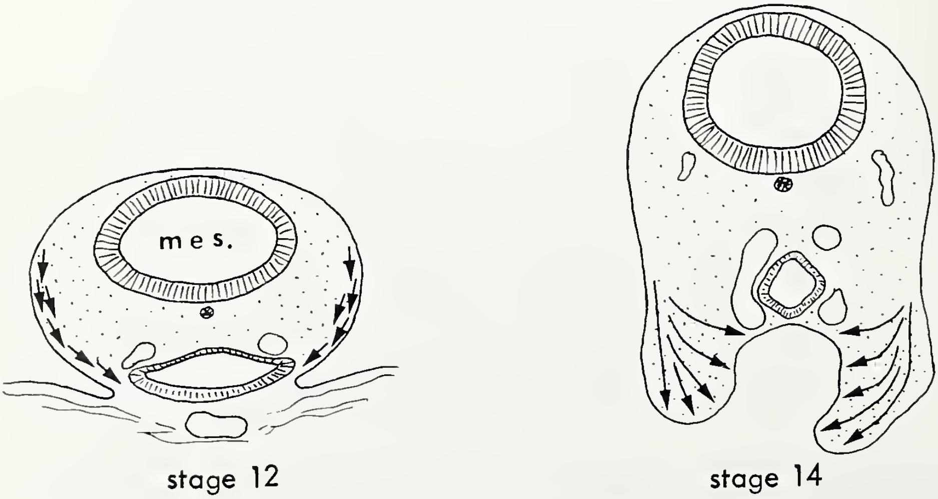

Figure 2–14. Summary of Area 3 early migratory patterns.

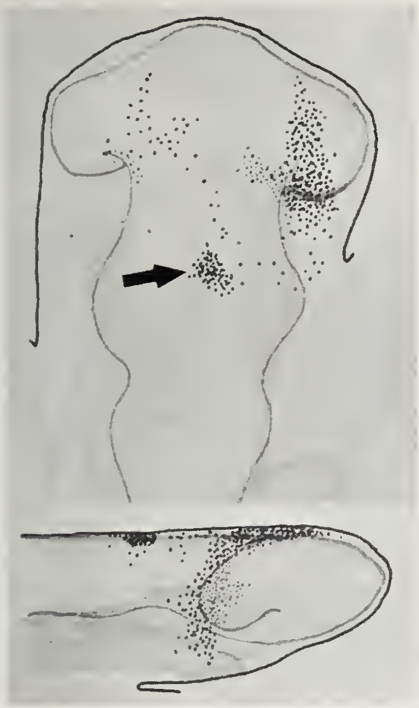

Figure 2–15. Reconstruction, stage 12⁻, orthotopic Area 2 transplantation (dorsal view, above; lateral view, below). Note the body of the graft (arrow) in the middle of the mesencephalon and the labeled cells which emigrated from it above and around the optic stalks.

3. Migratory patterns, Area 2

The initial migratory behavior of Area 2 neural crest cells is drastically different from that seen in more caudal areas. These cells, which are the first crest cells in the embryo to form, move rostrally along the dorsal midline of the mesencephalon, then turn laterally and are found principally over the future optic stalks by stage 11 (Figure 2–15). Shortly thereafter many Area 2 crest cells move around the caudal surface of the constricting optic stalks (Figures 2–15 and 2–16). The remainder of the population forms mesenchyme above and, later, rostral to the eye. This later deployment of Area 2 neural crest cells is shown in Figure 2–12 and summarized in Figure 2–17.

Nearly all of the mesenchymal and connective tissue elements in the avian embryo rostral to the junction of the mesencephalon and diencephalon are derived from neural crest cells, including parts of the cerebral meninges, the outer eye coats, structural components of the rostral chondrocranium, and many facial elements. The later development of these crest derivatives is discussed in greater detail in Chapter 3 of this symposium.

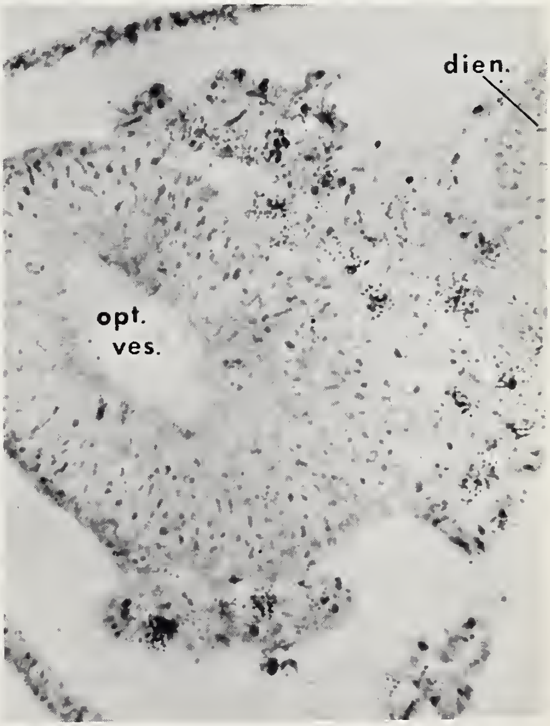

Figure 2–16. Radioautograph, Area 2, stage 12⁻, showing labeled cells above and below the optic vesicle (*opt. ves.*), and between the vesicle and the diencephalon (*dien.*).

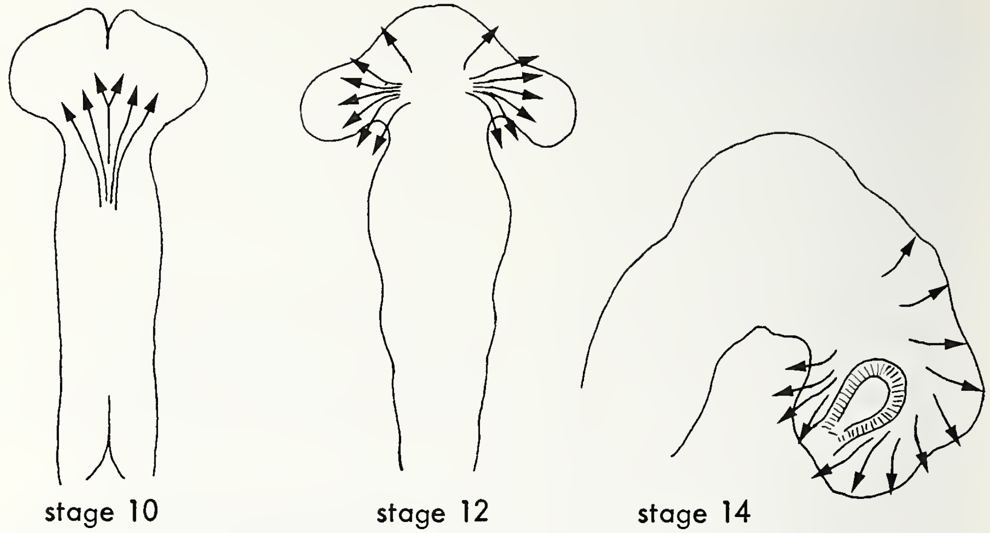

Figure 2–17. Summary of Area 2 early migratory patterns.

V. ANALYSIS OF NEURAL CREST MIGRATORY BEHAVIOR

One of the central and most enigmatic concepts in developmental biology is the determination problem.[9,13,31] When, and by what mechanisms, is the fate of a cell or tissue irreversibly fixed? Many examples are known in which a series of sequential interactions precede the stable expression of a given cellular phenotype; [19] this is called progressive determination. On the other hand, embryonic systems in which parts are determined very early in development have been reported.[2,53] With respect to neural crest development it is essential to know whether the cells are pluripotential as they first emigrate from their origin, or are already committed (determined) to a specific migratory pattern and later fate. If each neural crest cell within a given area is pluripotential, then complex migratory patterns such as those described in the previous section must be formed as a result of local environmental factors influencing the motile crest cells.

1. Available Evidence

Support for the pluripotentiality hypothesis comes from experiments performed by Detwiler [6] on urodele embryos. Observing that the population of trunk crest cells became subdivided segmentally only upon entering the previously segmented somitic mesenchyme, Detwiler posed the following question: Is the metameric pattern of the trunk neural crest population causally related to that of the somites, or are the two events coincidental?

To resolve this problem Detwiler replaced two large cervical somites with three smaller thoraco-lumbar somites prior to the onset of neural crest cell migration. As the wave of crest cells entered the somitic mesenchyme they reflected the new metameric pattern of the region, forming three presumptive spinal ganglion condensations instead of the normal two. Thus the pattern of condensation of these ganglia is established in response to some influence of the segmented somitic mesenchyme. In addition Weston [46] found that in chick embryos the neural crest cells condense upon entering somitic mesenchyme regardless of the direction from which they enter, which corroborates observations previously made by Hörstadius and Sellman [18] on the migrations of urodele cephalic neural crest cells following heterotopic transplantation. These experiments suggest that the migratory behavior of neural crest cells is not precisely determined within each cell prior to its emigration, but rather is influenced by the environment into which these cells migrate.

Further evidence suggesting pluripotentiality with respect to migratory behavior comes from the transplantation studies of Weston and Butler [51] in which a segment of spinal cord was removed prior to the formation of the neural crest and in its place was grafted a segment of "old" spinal cord, one from which most neural crest cells had already emigrated. Nonetheless, those neural crest cells which did form from the graft emigrated into the "young" environment and displayed the normal array of migratory patterns. This evidence eliminates the possibility which had previously been suggested [12,44] that there exist temporal differences within the neural crest population.

Evidence to the contrary, that the migratory behavior of some neural crest cells is independent of specific environmental influences, comes from a series of investigations performed by Twitty [40,41] on the migration of presumptive melanoblasts in urodeles. He observed that in two species of California salamanders, *Taricha torosa* and *Taricha rivularis* the presumptive melanoblasts initially distributed themselves evenly throughout the epidermis of the dorsal part of the embryo, and then began the synthesis of melanin. However, those of *T. torosa* secondarily aggregated, forming a longitudinal band of pigment-producing cells opposite the dorsal margin of the somites, while in *T. rivularis* they remained dispersed. Twitty then asked the question: Is this aspect of neural crest migratory behavior dependent upon properties inherent to the crest cells, and independent of specific environmental factors, or is it affected by influences extrinsic to the neural crest population? In each of two sets of experiments, one in which *T. rivularis* neural folds were replaced with *T. torosa* neural folds, and another in which neural crest cells from the two species were placed *in vitro*, the species-specific behavioral pattern was reproduced. The *T. torosa* crest cells moved apart and then secondarily aggregated, either beneath the epidermis of *T. rivularis* or *in vitro*. This is a clear case in which the migratory behavior of a particular population of neural crest cells has been proved to be independent of specific environmental influences.

2. Heterotopic Transplantations Within Cranial Areas

To resolve the problem of the control of neural crest migratory behavior within the pre-otic regions of the chick embryo, a series of heterotopic transplantations between Area 2 and Area 4 were performed. In all cases the donor tissue was labeled with ^{3}H-thymidine. If the crest cells are pluripotential and their migratory patterns are the result of environmental influences, then the resulting host embryos should be indentical to those which received orthotopic

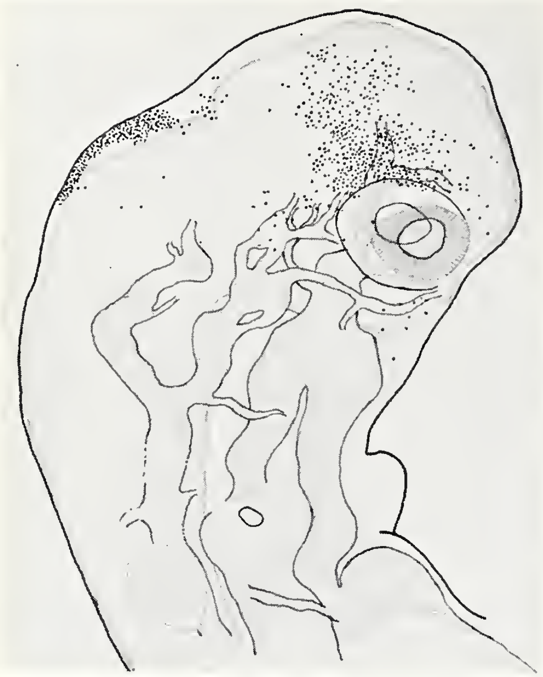

Figure 2–18. Reconstruction, stage 14⁻, heterotopic Area 4 into Area 2 transplantation. Note normal distribuiton of Area 2 crest cells.

transplantations. On the other hand, if the potentiality of crest cells is restricted and their migratory behavior directed by influences intrinsic in the cells, then the host embryos should have abnormal distributions of grafted neural crest cells.

Figures 2–18 and 2–19 indicate that neural crest cells emigrating from an Area 4 neural fold which had been grafted into Area 2 mimic the normal Area 2 migratory pattern, moving rostrally over the optic stalk. The later movements of heterotopically transplanted cells is shown in Figures 2–20 and 2–21. These crest cells migrated around the optic cup and, at stage 15, are found surrounding Rathke's pocket (presumptive adenohypophysis, Figure 2–21a) and contributing to the formation of the maxillary process (Figure 2–21b). In addition, labeled cells are found around the outer rim of the optic cup; neural crest cells from this position normally invade the primary corneal stroma at later stages (see section VI).

The reciprocal heterotopic transplantations of Area 2 into Area 4 yielded even more dramatic results. Area 2 crest cells, which normally would never condense into a presumptive sensory ganglion,

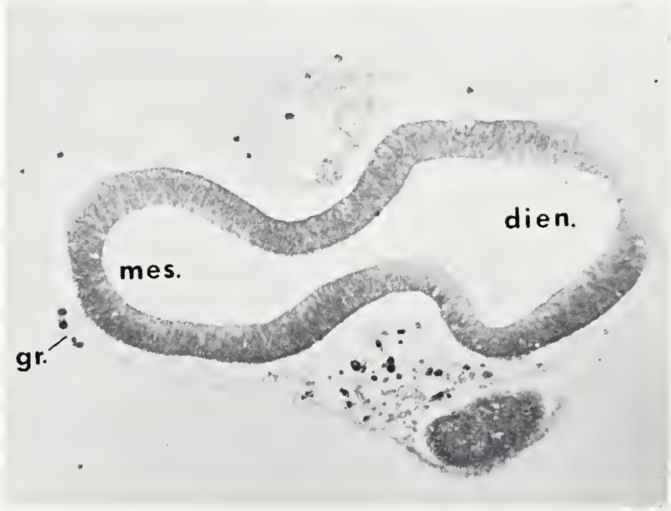

Figure 2–19. Radioautograph, stage 14⁻, showing distribution of Area 4 crest cells in Area 2 following heterotopic transplantation. The position of the graft (*gr.*) is indicated. Section from embryo reconstructed in Figure 2–18.

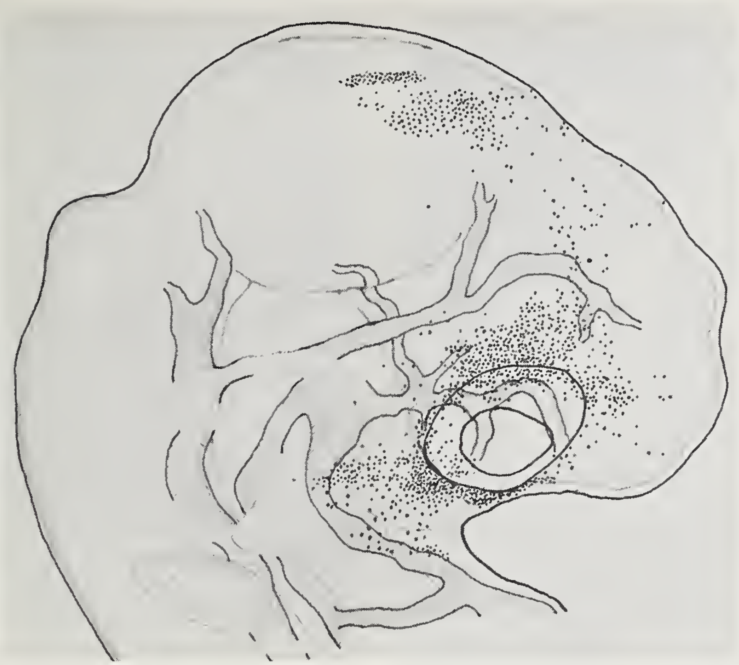

Figure 2–20. Reconstruction, stage 15, Area 4 into Area 2 heterotopic neural fold transplantation. The pattern of crest cell positions is indistinguishable from that seen following orthotopic transplantation (compare with Figure 2–12).

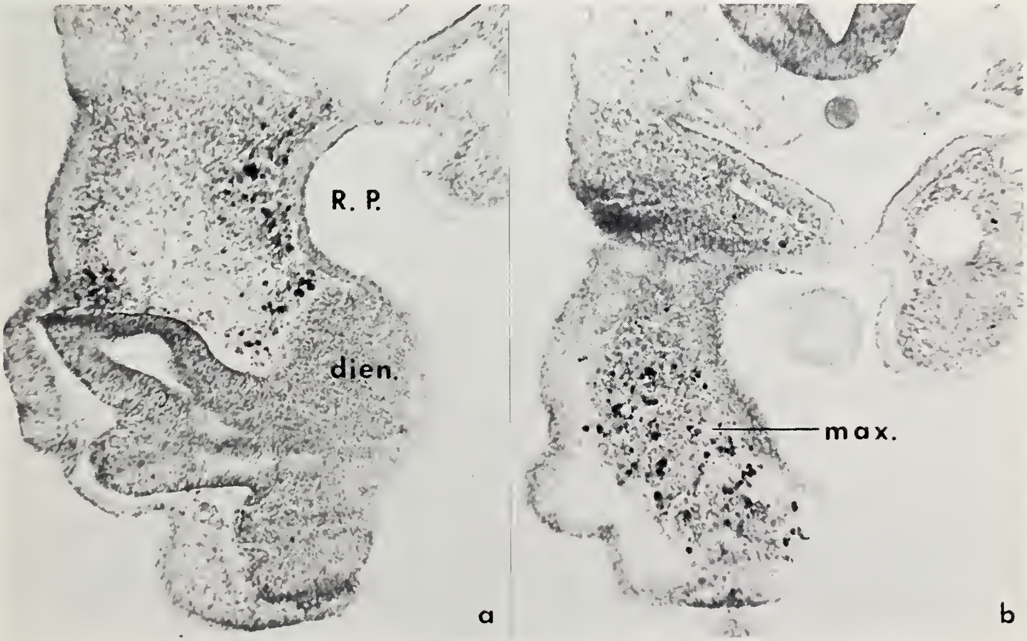

Figure 2–21. Radioautographs, stage 15. Sections through reconstructed embryo, Figure 2–20. a) shows labeled cells adjacent to the diencephalon (*dien.*) and Rathke's pocket (*R. P.*). Note also the presence of labeled crest cells above the outer rim of the optic cup. b) illustrates the position of these heterotopically grafted cells in the maxillary process (*max.*).

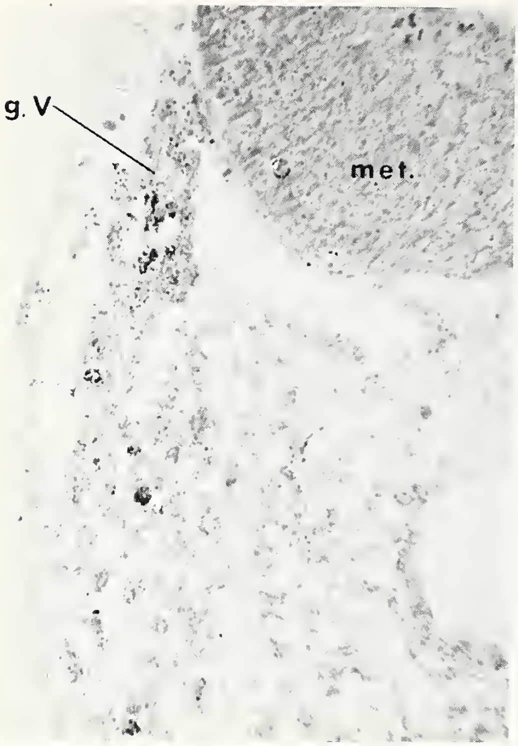

Figure 2–22. Radioautograph, stage 14. Section through reconstructed embryo, Figure 2–23. Demonstrates that Area 2 cells will, when grafted heterotopically into Area 4, migrate to and aggregate into a trigeminal ganglion (*g. V*).

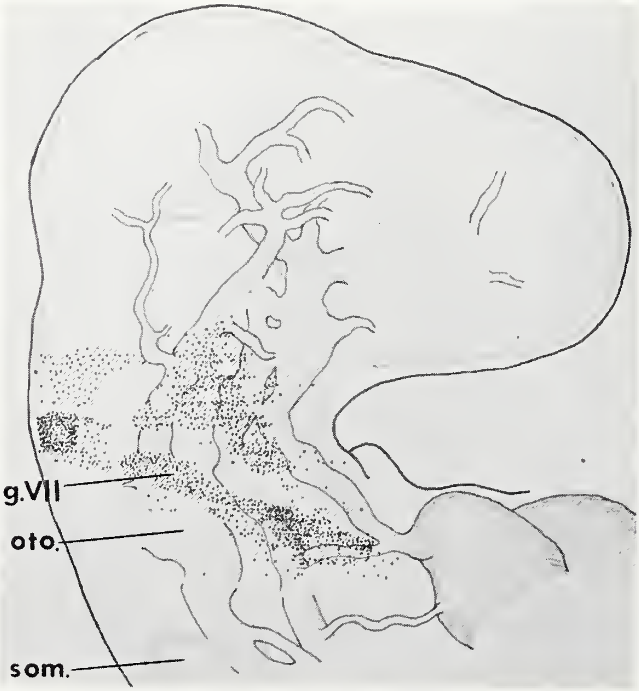

Figure 2–23. Reconstruction, stage 14, heterotopic Area 2 into Area 4 and Area 5 transplantation. Note the pattern of distribution of labeled cells, which is very similar to that seen in Figure 2–8, an orthotopic Area 4 graft.

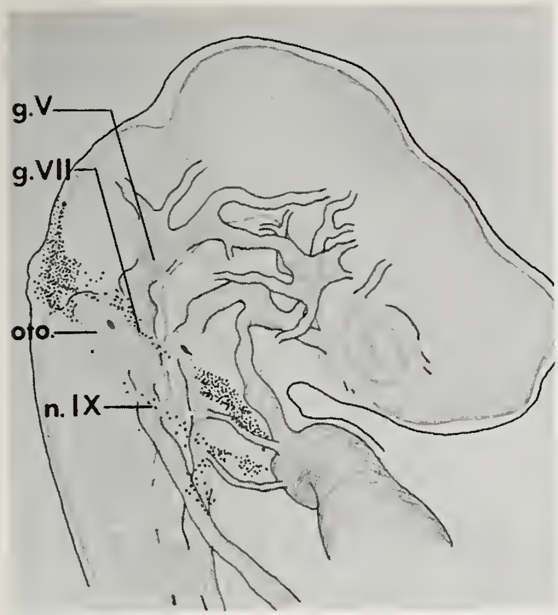

Figure 2–24. Reconstruction, stage 15. Heterotopic graft from Area 2 into Area 5. Some labeled cells migrated caudal to the otocyst (*oto.*), and are seen along cranial nerve IX (*n. IX*) and in the third visceral arch. The geniculate ganglion (*g. VII*) and hyoid arch are heavily labeled.

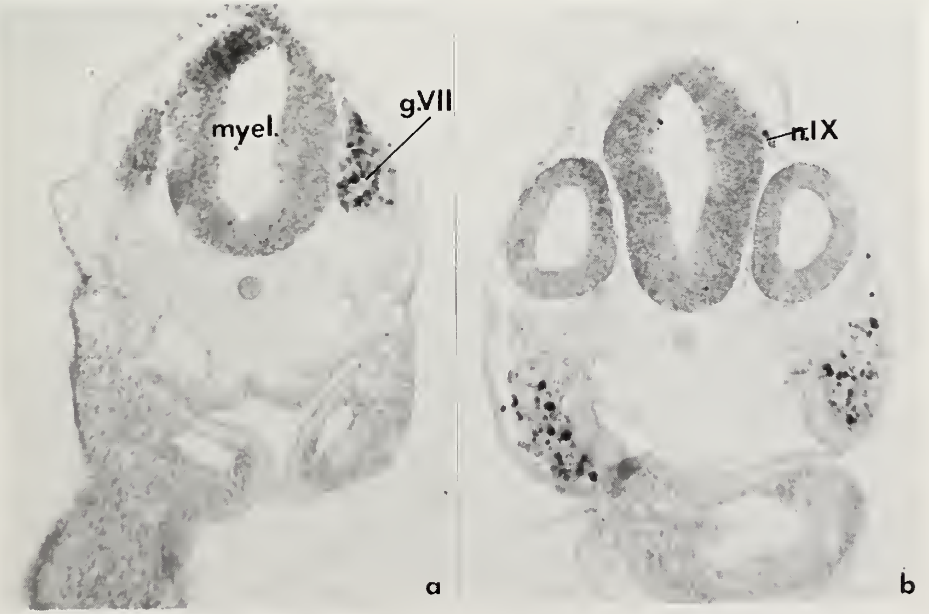

Figure 2–25. Radioautographs, stage 15. Sections through embryo reconstructed in Figure 2–24, a heterotopic Area 2 into Area 5 transplantation. a) indicates the presence of labeled cells in the geniculate ganglion (*g. VII*). b) illustrates the location of labeled neural crest cells along the root of the ninth cranial nerve (*n. IX*) and bilaterally in the hyoid visceral arches.

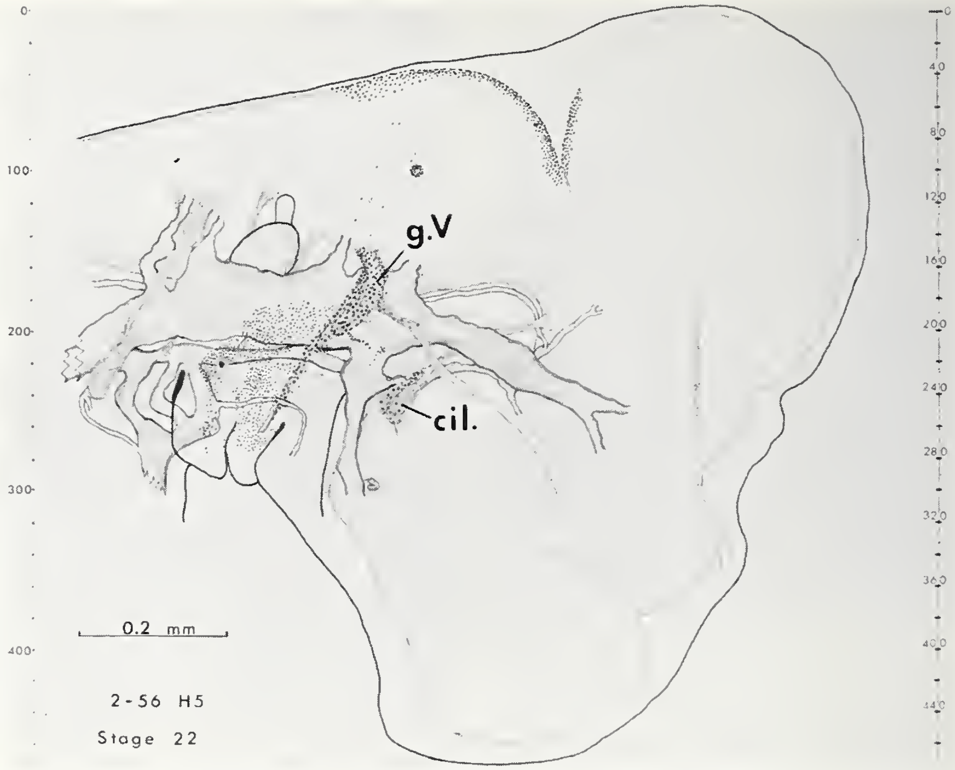

Figure 2–26. Reconstruction, stage 22 (4 days), following heterotopic transplantation of Area 2 neural fold into Area 4. Note the presence of labeled cells in the first and second visceral arches, the trigeminal ganglion (*g. V*) and associated nerves, and the ciliary ganglion (*cil.*).

will do so when placed in Area 4 or Area 5 (Figures 2–22 through 2–25). Furthermore, these heterotopic neural crest cells will penetrate the visceral arches in a manner identical to that seen following orthotopic transplantation. The development of these embryos has been followed through four days of incubation (Figure 2–26), and no significant deviation from normal migratory behavior has been found. These results indicate that cephalic neural crest cells are pluripotential with respect to their migratory behavior, and that the patterns of their migrations are largely under the control of local environmental influences.

3. Heterotopic Transplantations Between Trunk and Head Regions

Numerous investigators have described severe abnormalities which result from replacing cephalic neural crest cells with those of the trunk in amphibian embryos.[3,18,28] These abnormalities include reduction or absence of cartilagenous elements of the visceral arches and of the anterior chondrocranium, odontoblasts, and other neural crest-derived skeletal elements. However, it is not known whether these deficiencies result from an inability of trunk crest cells to migrate through the head regions, as suggested by Chibon,[3] or a failure of these heterotopic cells to respond to specific inductive stimuli.

To resolve this problem in the chick, Area 4 neural fold tissue was replaced with ^{3}H-thymidine labeled brachial neural fold tissue. The results are shown in Figures 2–27 through 2–29. These heterotopic crest cells migrate into the head tissues, and do so in a manner indistinguishable from that seen in a normal embryo. Despite the absence of defined somites in this region (small preotic somites, which give rise to extrinsic

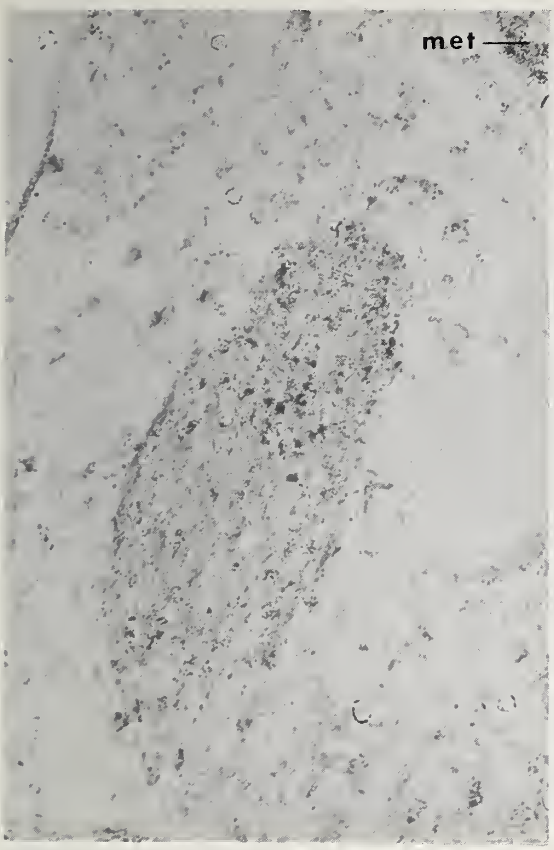

Figure 2–27. Radioautograph, stage 18$^+$ (3 days). Illustrates the presence in the trigeminal ganglion of crest cells derived from a piece of trunk neural fold which had been transplanted into Area 4. Note the concentration of labeled cells in the medial part of the ganglion (closer to the metencephalon, *met.*). The lateral part of the ganglion is formed mostly from cells derived from the epidermal placodes.

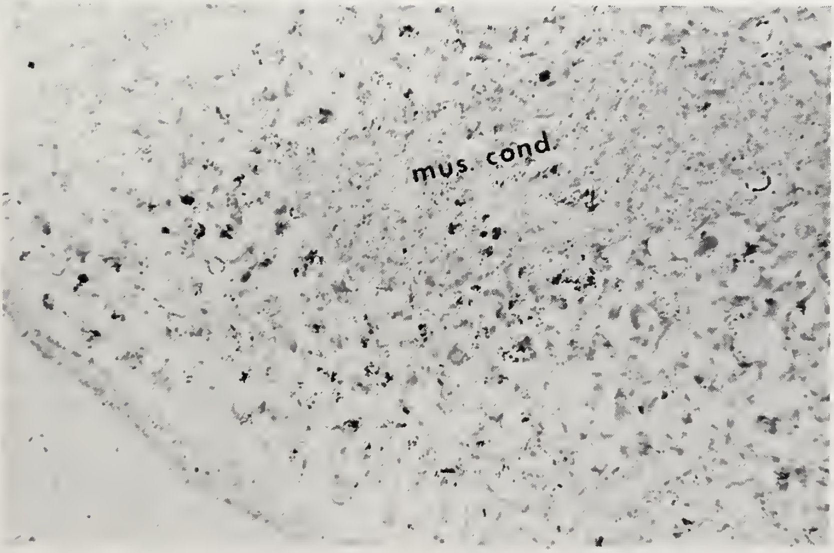

Figure 2–28. Radioautograph, stage 18$^+$. Section from same embryo as Figure 2–27. Shows the presence of heterotopically transplanted trunk crest cells in the first visceral (mandibular) arch surrounding the mesodermal muscle condensation (*mus. cond.*).

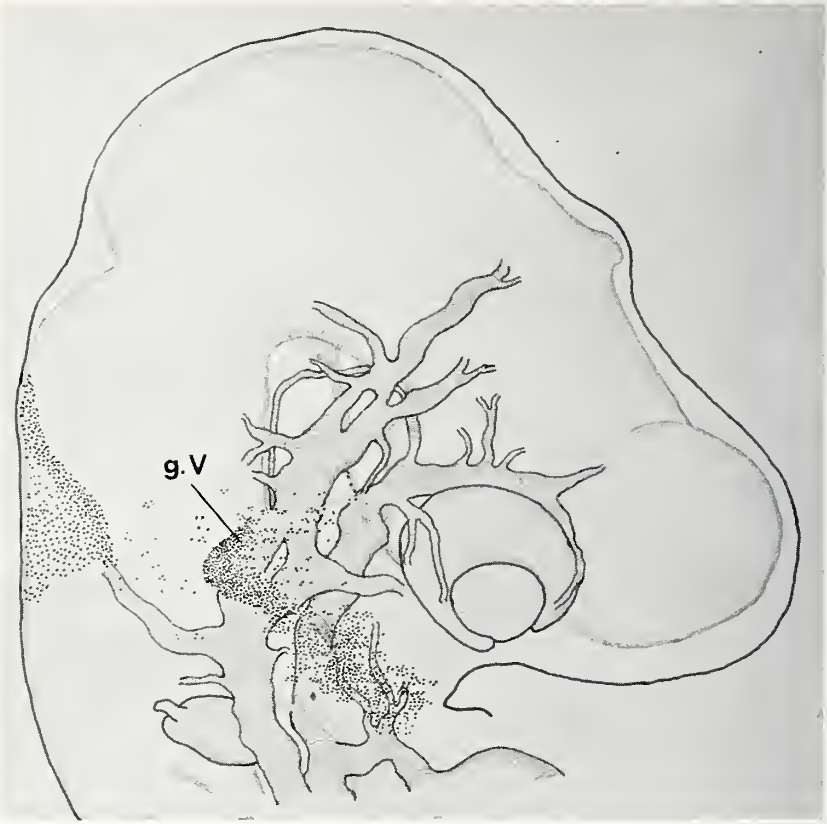

Figure 2–29. Reconstruction, stage 18^{+}. Same embryo as Figures 2–27 and 2–28, a heterotopic transplantation of brachial level neural fold into Area 4. The pattern of neural crest distribution in this embryo is indistinguishable from that seen following orthotopic Area 4 transplantation.

eye muscles, form at later stages), some trunk crest cells responded to local environmental influences and condensed to form a morphologically normal presumptive trigeminal ganglion. Moreover, the topographical distribution of neural crest and placode cells within the ganglion is normal (Figure 2–27), even though trunk crest cells normally never interact with placode cells. Figure 2–28 illustrates the presence of grafted trunk neural crest cells in the mandibular arch mesenchyme, surrounding the condensed mesodermal muscle primordium.

Due to progressive dilution of the label during cell division it is not feasible to maintain the hosts until chondrogenesis is histologically demonstrable. Thus it is still not known whether trunk crest cells will form cartilage following their entry into visceral arches. Experiments to test this using a permanently replicating marker are currently in progress.

The results of heterotopic transplantations clearly indicate that the migratory behavior of chick neural crest cells in the cephalic regions is strongly influenced by local environmental factors. Moreover, these experiments reveal that all neural crest cells, regardless of their region of origin, have the capacity to recognize and respond to specific environmental influences emanating from other regions, indicating that they possess no inherent restrictions as to their migratory behavior. Whether these cells are equally pluripotential with respect to their later cytodifferentiation remains to be examined.

VI. ENVIRONMENTAL INFLUENCES

Recent experiments have begun to reveal the nature of some specific environmental influences which direct the migratory behavior of neural crest cells. For example, Weston [48,49] has suggested that aggregation of neural crest cells to form spinal ganglia is the result of changes in adhesive properties of the crest cells. Further, based on *in vitro* analyses, he proposes that nerve growth factor [24] present within somitic mesenchyme might be the environmental influence responsible for this component of neural crest migratory behavior. The molecular basis of such cell-cell interactions, which have been found to play an essential role in many morphogenetic processes [37,39] including the migrations of presumptive neuroblasts in the developing central nervous system,[29] are not yet resolved.

One system in which the specific environmental influences have been identified is the developing avian cornea. Prior to the sixth day of incubation the cornea consists of an outer corneal epithelium which secretes a collagenous matrix [7] called the primary corneal stroma.[15] The collagen fibers in the acellular matrix are arranged in an orthogonal pattern with no angular displacement.[38] Between days six and ten this stroma is invaded by mesenchymal cells derived from the neural crest. The primary stroma acts as a scaffold and the crest cells become oriented in a similar pattern. It is these cells which begin to secrete the secondary (adult) stroma on the eighth day of development.

Shortly before being invaded by mesenchymal cells, the primary stroma swells due to hydration. Also at this stage there is an elevation in the level of synthesis of the glycosaminoglycan hyaluronate.[35] Beginning on the tenth day of development the cornea becomes more compact and undergoes dehydration.[15,38] Concomitantly there is a dramatic rise in the enzymatic level of hyaluronidase, and a decrease in hyaluronate synthesis.[35]

These data alone do not justify the proposal of a causal link between molecular development of the primary stroma matrix and the migratory behavior and cytodifferentiation of corneal mesenchyme cells. However, similar patterns of hyaluronate biosynthesis occur during axial skeletogenesis and limb development and regeneration.[32,34] Furthermore, *in vitro* studies indicate that hyaluronate prevents aggregation and promotes cell movement of presumptive chondroblasts, and at the same time prevents their further cytodifferentiation.[36] These effects are prevented by the presence of thyroxine, growth hormone, or adenosine 3′,5′-monophosphate.[33] In the light of this evidence, together with the established role of collagen in the development of other cell strains,[14] it seems likely that there is a causal relation between the formation of a collagenous matrix with varying levels of hyaluronate and the migratory behavior and cytodifferentiation of this population of neural crest cells.

Thus, in one situation the elusive environmental influences are beginning to be defined. Hopefully such analyses will lead to a better understanding of the control of neural crest migratory behavior and its relation to the mechanisms of cytodifferentiation.

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DISCUSSION

STEINER: I would like to ask whether you know if this migration is an active or a passive one. In case you do believe that there is a chemical factor which is involved in this oriented migration, then these cells must migrate by some chemotactic mechanism. Do you have any evidence of such a mechanism?

NODEN: These cells are actively migratory, as has been shown by numerous *in vitro* observations.

STEINER: By pseudopodia?

NODEN: Yes, at least in part. To my knowledge they have not been carefully examined for the presence of ruffled membrane regions and other structures characteristic of motile cells.

In regard to the second part of your question, we know from the *in vitro* experiments of Twitty and Niu [1] that diffusible chemical factors can affect the migratory behavior of amphibian neural crest cells. However, it is not clear whether such mechanisms occur *in situ*. Certainly some sort of cell:cell recognition and interaction must be in operation for the crest cells to behave in so highly patterned a manner. I would refer you to a recent review by Weston [2] in which these problems are more fully discussed.

THACH: As neural crest cells migrate outward, they begin to differentiate. If you were to take these cells and transplant them back into the crest, would they then revert back to an undifferentiated form capable of migration?

NODEN: Your question touches on two important concepts in development; namely, the degree of stability of a determined cell and the progressive nature of determination. I interpret my results as indicating that the neural crest population is pluripotential prior to the onset of migration. An alternative interpretation would be that these cells are in some way regionally specified but not irreversibly so. Then, when placed in a different environment, their previous commitment is altered by new environmental influences.

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[1] Twitty, V.C. and M.C. Niu (1954) The motivation of cell migration studied by isolation of embryonic pigment cells singly and in small groups *in vitro*. J. Exp. Zool. *125*:541–574.

[2]. Weston, J. (1971) Neural crest cell migration and differentiation. In "Cellular Aspects of Growth and Differentiation in Nervous Tissue" *UCLA Forum in Med. Sci.*, D. Pease Ed. *14*: 1–19.

I have not attempted the experiment which you suggest primarily because it would be very difficult to excise intact a pure neural crest population suitable for transplantation into the neural fold region of a younger embryo. Perhaps a better approach to this problem is that currently being used by Newsome and Coulombre at the National Eye Institute and Weston at the University of Oregon; namely, cloning neural crest cells at various stages of their development.

JOHNSTON: In the trunk region, much smaller numbers of neural crest cells are formed and migrate than in the head region. Yet it appeared to me that when you transplanted trunk crest into the head you had almost the same frequency of labelled cells in the ganglia and in the visceral arch region as in the embryo in which you had transplanted labelled head crest to this region.

NODEN: We know from ablation studies that if you take out a small region of the neural crest population the initial population size is restored quickly as the result of an increase in the division rate of cells adjacent to the wound. A similar phenomenon might occur following my trunk-into-head neural fold transplantations.

I do have some very interesting cases in which transplanted trunk crest cells contributed to cranial ganglion formation but did not migrate into the visceral arches. Yet the arches appeared to be normally developed. These results can be explained on the basis of regional differences in the timing of onset of neural crest migration. It takes approximately four to six hours for the entire complement of metencephalic crest cells to leave their area of origin, whereas a significantly lesser number of trunk crest cells require over three times that amount of time to emigrate from the dorsal midline of the spinal cord (Weston and Butler, 1966). I believe that the occasional absence of transplanted trunk crest cells in the visceral arch regions is the result of their late onset of migration. Thus, the visceral arch regions have already been filled by adjacent cephalic crest cells prior to the potential arrival of transplanted cells.

GREULICH: In that same vein, when you have transplanted trunk neural crest to the head, do those cells then move at the same rate as the normal head crest, or do they migrate more slowly? Is the rate of migration predetermined?

NODEN: This is an important factor about which, unfortunately, I have little information. Since my orthotopic and heterotopic hosts are essentially indistinguishable, I would not think that regional differences in migratory rate exist. What I would very much like to know is whether the pre-migratory neural crest population in a specific area is made up of cells programmed to migrate at different rates.

GASSER: In the primate embryo, there is a considerable number of mesodermal cells in the future head region between ectoderm and endoderm that, apparently, are derived from the primitive streak. What is the condition in the chick and what happens to these cells?

NODEN: Let me answer you very briefly since Dr. Johnston will be discussing this in more detail in his presentation.

The prechordal axial and paraxial mesoderm forms the ventral and ventrolateral elements of the chondrocranium and vascular tissues. Most other connective tissue elements rostral to the middle of the mesencephalic region are derived from the neural crest, while elements in the more posterior parts of the head are of either mesodermal or mixed origin. Thus the neural crest and mesodermal cells share many histogenic potentialities, the differences between them being regional.

SAMEROFF: It is interesting that there seem to be no structural differences as a result of your tissue transplants. The resulting anatomy appears to be the same as if no transplant had occurred. Does this result also apply to functional differences? That is, do the animals that have had transplants show no differences in behavior after they are born, compared with those that have not had transplants?

NODEN: My results indicate only that pre-migratory neural crest cells are pluripotential with respect to their migratory behavior. At this time I cannot say whether the same is true with respect to their cytodifferentiation. For example, I have not allowed hosts to develop to stages at which visceral arch chondrogenesis occurs, thus I do not know whether a trunk neural crest cell which normally never would differentiate into a chondroblast can do so when transplanted into the head region. These experiments are in progress at this time.

I have not made any observations of the behavior of these birds. In view of the fascinating results of Székely [1] who performed placode transplants in amphibians, behavioral analyses of my hosts could be most interesting.

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[1]. Székely, G.: Functional specificity of cranial sensory neuroblasts in urodela. *Acta. Biol. Aca. Sci. Hung.* 10:107–116, 1959.

Chapter 3

AN EXPANDED ROLE OF THE NEURAL CREST IN ORAL AND PHARYNGEAL DEVELOPMENT

M. C. JOHNSTON, A. BHAKDINARONK AND Y. C. REID

Since the last Symposium, when we presented material on the broader subject of the origins of facial tissues, there have been a number of advances in knowledge about the neural crest and its relation to facial development. Some of these are reviewed by Dr. Noden in Chapter 2; his material deals primarily with the mechanisms of migration.

The tritiated thymidine marker used by Dr. Noden is excellent for following early cell migration since the number of cell divisions in early embryonic development is very small. It gives a very accurate picture of the timing and pattern of cell migrations since there is no possibility of inaccuracies introduced by variations in developmental rate such as those possibly introduced by the cross-species transplants. However, for following the fates of graft cells in late differentiating tissues, the tritiated thymidine marker may become so diluted that it becomes undetectable.

A recent significant advance has been the development, in France, of a new cell marker system (LeDouarin and Barq,[12] and LeDouarin,[11]) which permits one to follow the history of embryonic cells indefinitely. This cell marker consists of a large mass or masses of unwound chromosome material found in the nuclei of cells of the Japanese quail (see Fig. 3–3). This mass of condensed chromatin is usually found in the center of the nucleus, in contrast to the rather diffuse chromatin pattern found in the cells of the chick embryos which serve as hosts for quail embryo grafts. Unlike the tritiated thymidine marker, the quail chromatin replicates with each cell division. Therefore, one can follow the migration of these cells and their eventual fate without regard to the number of cell divisions.

The transplantation technique used for grafts made between chick and quail embryos is illustrated in Figure 3–1. It is basically the same as the technique described by Dr. Noden for his tritiated thymidine marker experiments.

In this chapter, the derivatives of the neural crest in the oral and pharyngeal regions will be considered. These derivatives may be divided into three major groups: mesenchymal, neural and endocrine.

MESENCHYMAL DERIVATIVES

There is now a large amount of evidence indicating that embryonic mesenchyme, the forerunner of "skeletal and connective tissues", originates

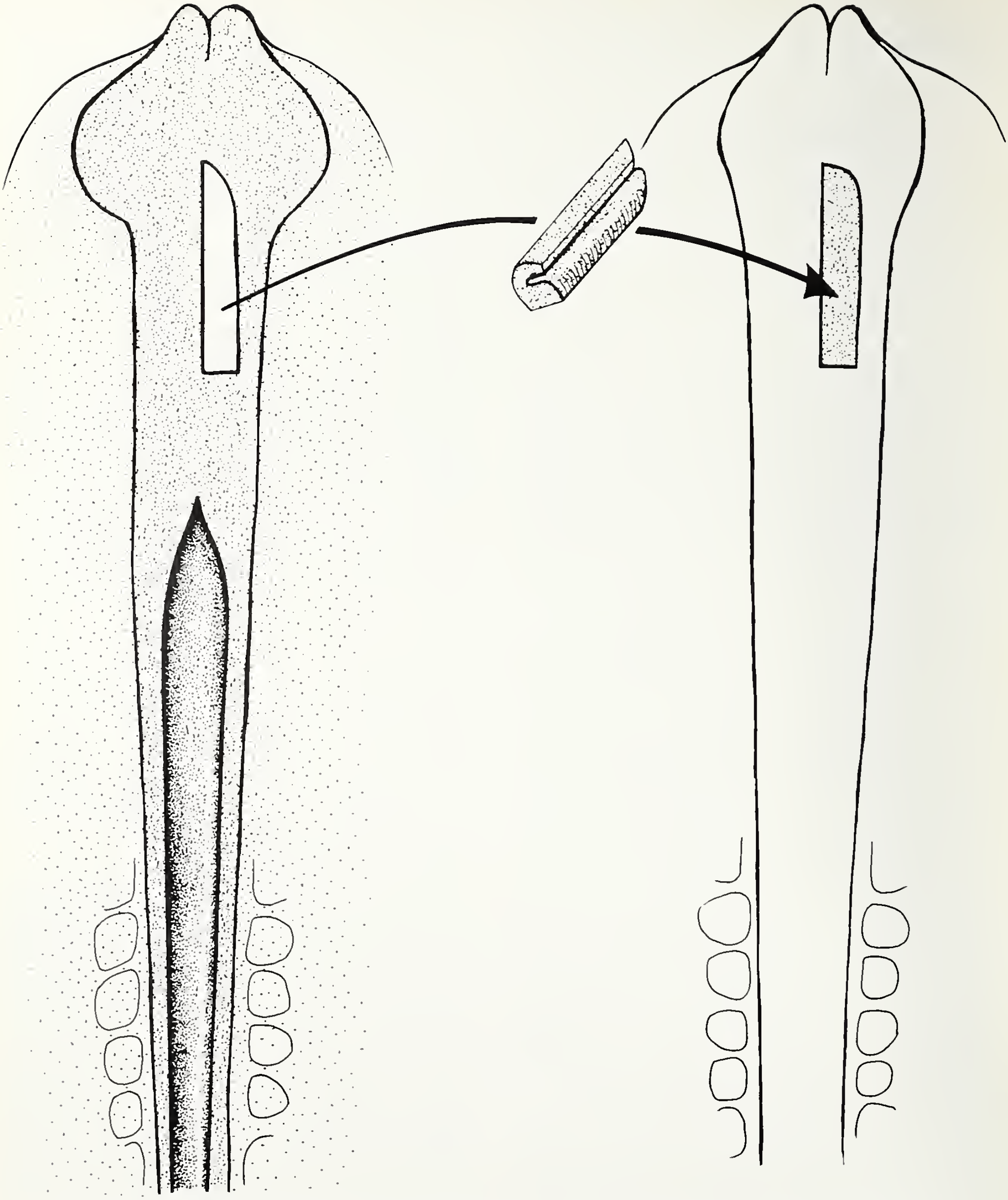

Figure 3–1. Graft transfer between donor quail embryo (stippled) and chick host. In this example, the crest material is contained in a unilateral neural fold graft.

both from mesoderm and from the cranial neural crest. Any of the derivatives of mesodermal mesenchyme can be formed by mesenchyme derived from crest. These include a large variety of tissues such as cartilage, bone and general connective tissue. The quail-chick transplant studies have been invaluable in determining precisely the relationships between mesenchymal tissues derived from quail neural crest grafts and mesenchymal tissues derived from the chick host mesoderm.

The relationships between tissues of crest and mesodermal origin remains essentially unchanged from the earliest stages following the completion of crest cell migrations (see Fig. 3–2). While, initially, no mesoderm is found in the upper facial region, cores of mesoderm are found within each of the visceral arches. The mesodermal cores are first

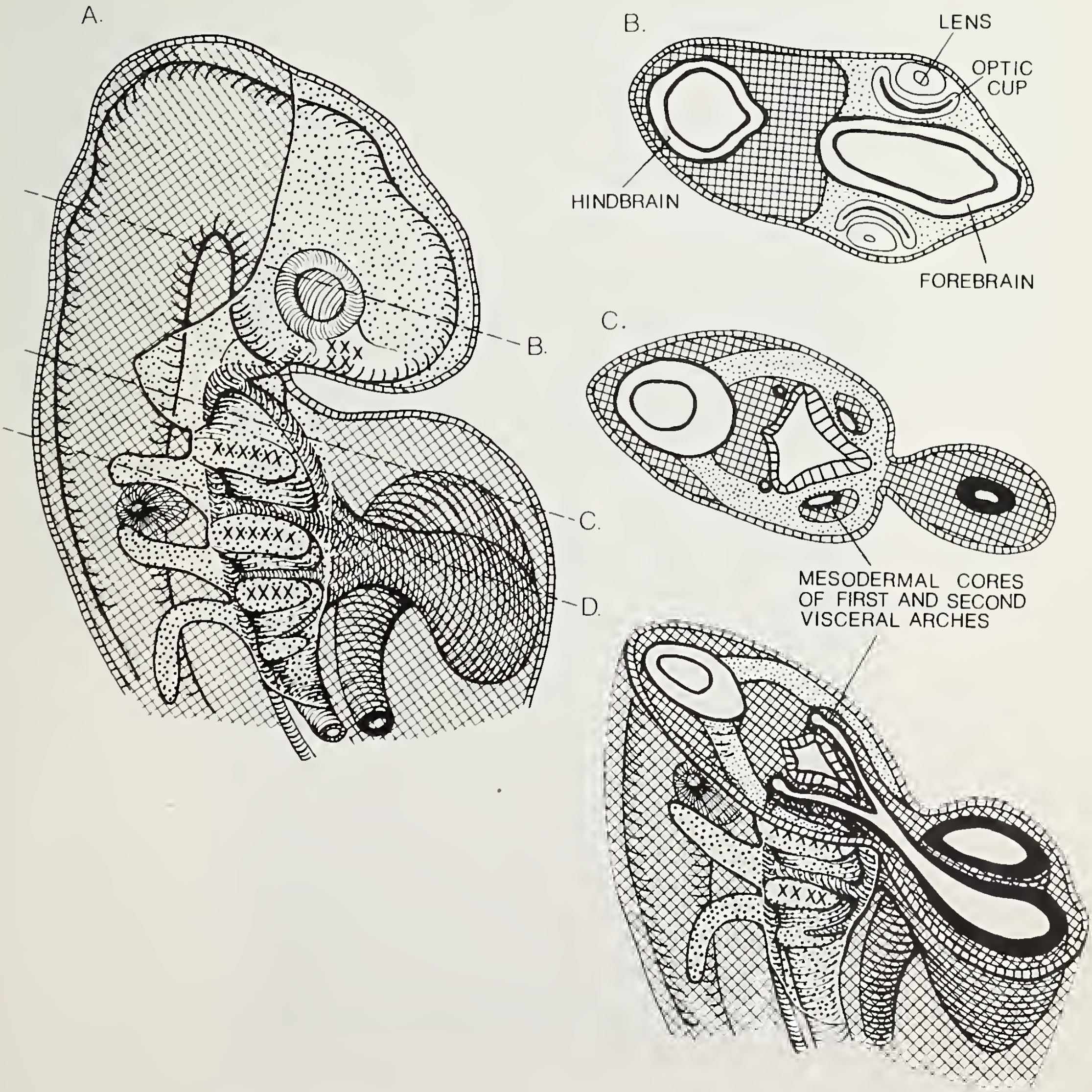

Figure 3–2. The contact relationships or interface between crest (strippled) and mesodermal (cross-hatched) mesenchyme is presented diagrammatically for avian embryos. Note the relations of crest cells to eye and forebrain (section B) and to the mesodermal cores and aortic arch vessels of the visceral arches (sections C and D).

concerned with the formation of blood vascular tissues while later they are concerned with the formation of skeletal muscle cells (Johnston and Listgarten, [10]).

In the earlier phases, the blood vessels migrate or grow out from the mesodermal cores and associate themselves with neural crest mesenchyme (see Fig 3–3). It appears that these mesenchyme cells eventually differentiate into perivascular cells (pericytes).

In later stages, prospective muscle cells migrate from the mesodermal core and become associated with the neural crest mesenchyme (see Fig. 3–4). In addition to forming the usual connective tissue elements of muscles, the crest mesenchyme appears to form the satellite cells which closely associate themselves with developing myotubes. Moss and Leblond[16] and others have provided evidence that these satellite cells eventually donate their nuclei to growing muscle cells. This is consistent with our observations in older embryos in which quail crest nuclei appear to be located within the muscle cell cytoplasm.

The earliest component of the skeletal

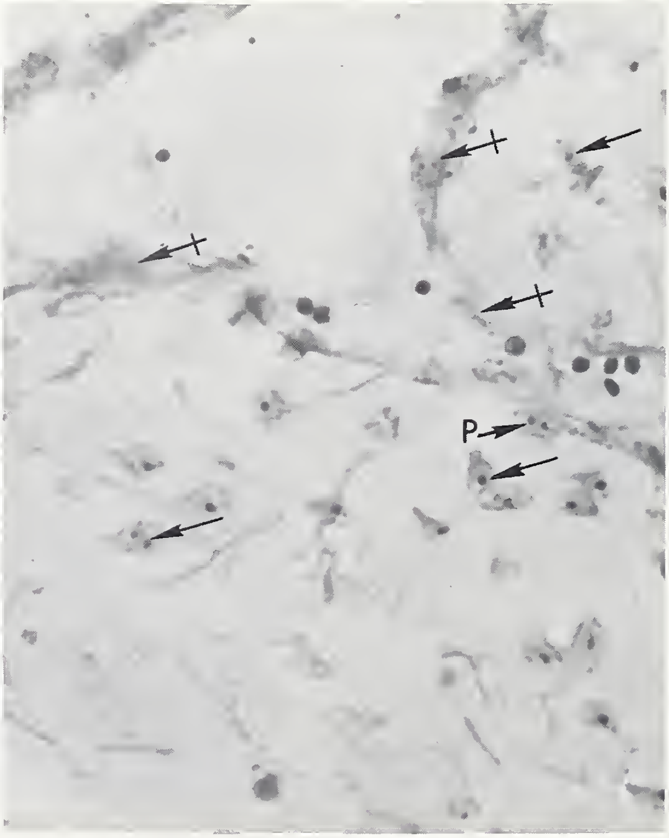

Figure 3–3. The large condensed chromatin mass or masses within the nuclei (arrows) of mesenchyme cells derived from a crest graft are easily distinguished from the diffuse chromatin pattern of the endothelial cell neclei (crossed arrows) of the chick host. The quail cell (P) tightly apposed to the endothelium may eventually differentiate into a pericyte.

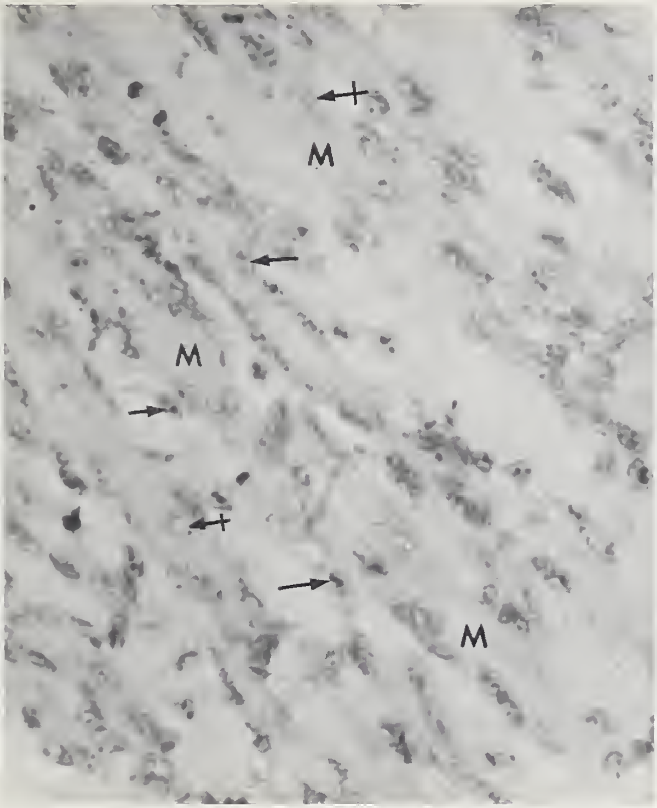

Figure 3–4. Visceral arch skeletal muscle of a chick host that had received a quail neural crest graft. Chick nuclei (crossed arrows) are seen within developing muscle cells (M) while the nuclei of the mesenchyme cells (arrows) contain quail chromatin indicating their origin from a quail neural crest graft. Some of the mesenchyme cells appear to be forming satellite cells which are tightly apposed to muscle cells.

system to be found in developing embryos is the cartilagenous skeleton. A number of investigators (e.g. Holtfreter, [6]) have shown that pharyngeal endoderm is responsible for inducing crest cells to form visceral arch cartilage (see fig. 3–12). That this cartilage is derived exclusively from neural crest is demonstrated in Figure 3–5. A similar pattern of development is found in each of the more posterior visceral arches.

Membrane bones, secondarily develop in association with these cartilages. Mandibular bone is illustrated in Figure 3–6. Since most of the bones developing in avian embryos appear to be homologous with those found in the human embryo, it is appropriate to extrapolate from one species to the other (see Fig. 3–7). Also illustrated in Figure 3–7 is the position of the crest:mesoderm interface within the dermal tissue. The quail-chick transplants show clearly that as the interface between mesoderm and crest is crossed, the origins of tissues in the different areas changes appropriately. The connective tissues supporting various organ systems such as the pituitary gland, the eye and the forebrain region are consequently also of neural crest origin.

While there is, of course, considerable

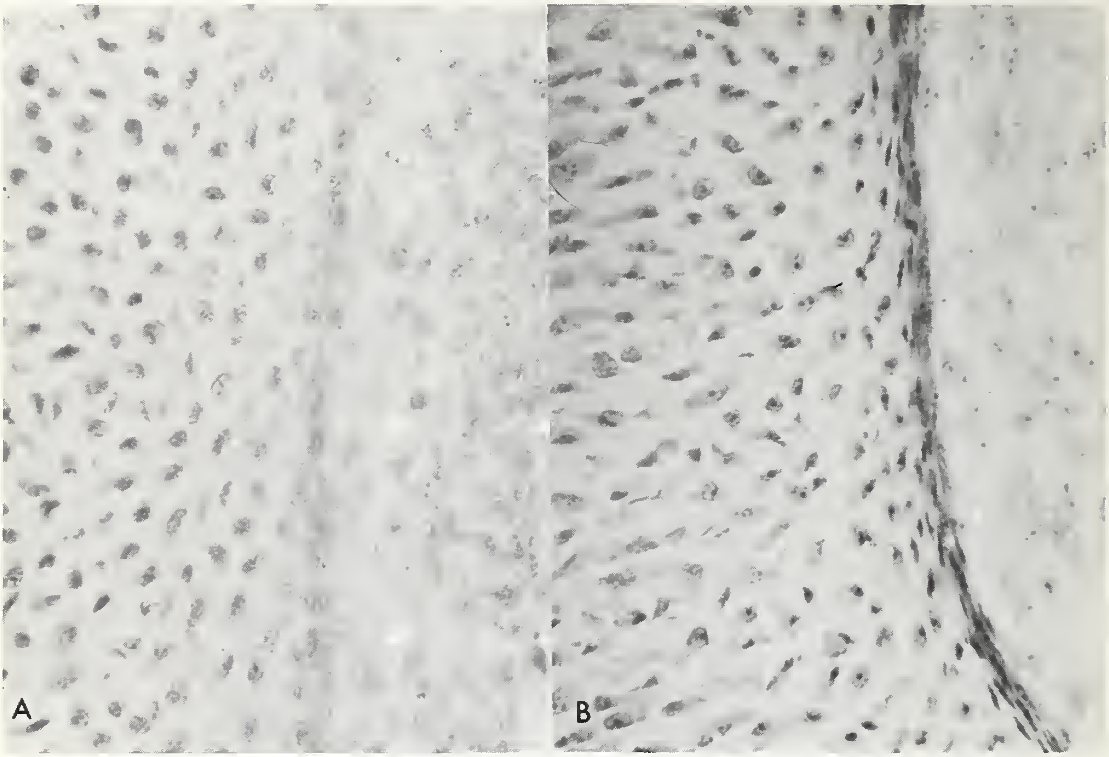

Figure 3–5. First visceral arch (Meckel's) cartilage on control (A) and graft (B) sides of a chick host that had received a unilateral quail neural fold graft. The dense chromatin masses in the chondrocyte nuclei of cells derived from the quail crest graft (B) are readily apparent.

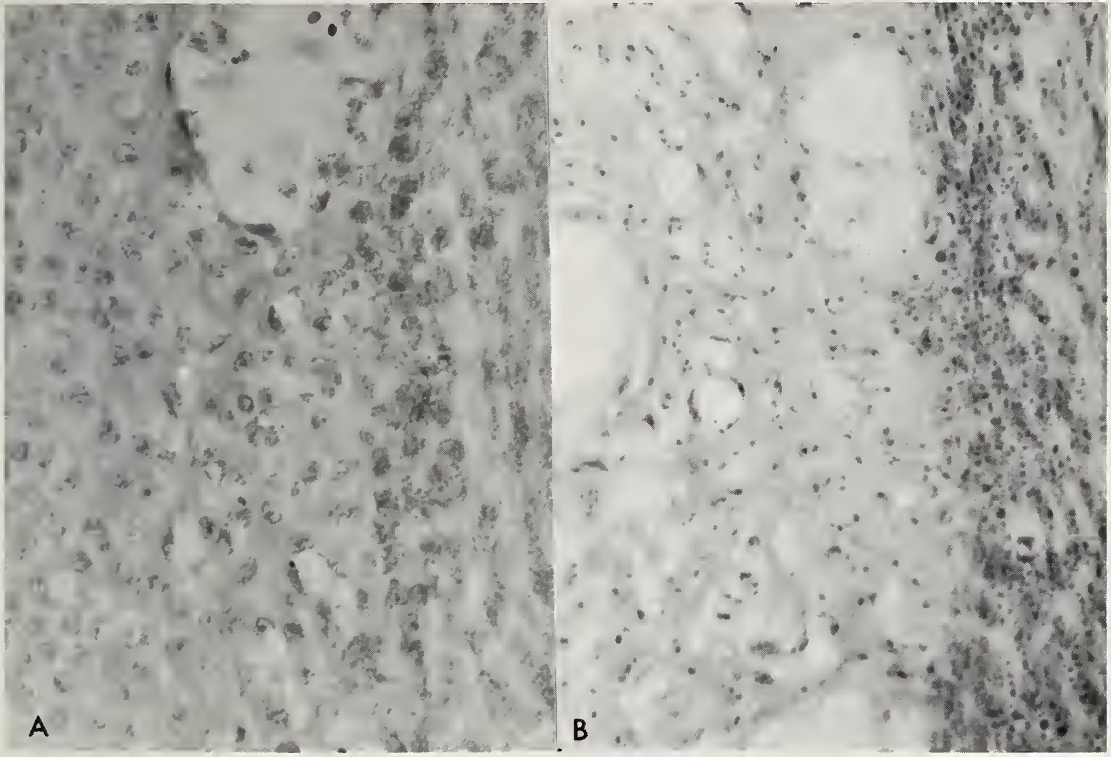

Figure 3–6. Bone of the mandibular arch developing outside Meckel's cartilage (same embryo as in Fig. 5). Bone on the graft side (B) contains quail crest cells.

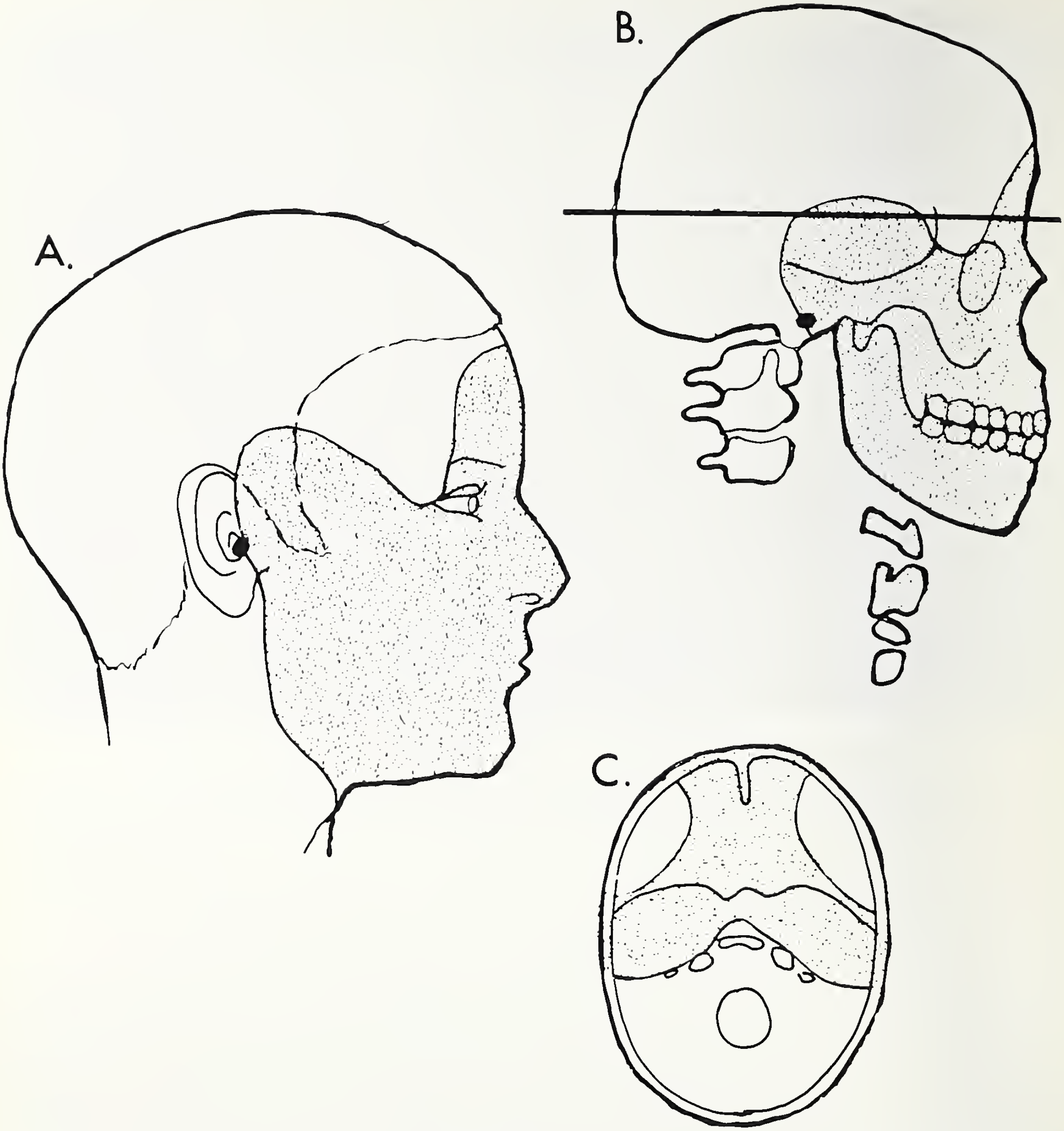

Figure 3–7. Extrapolation of results from avian studies to man based on homologies of individual bones and other criteria. A. Dermal tissues of crest (stippled) and mesodermal origins. Lateral view of skull (B) and view of floor of cranial fossae (C) showing bone estimated to be of crest (stippled) and mesodermal origins. (from Johnston[8]).

risk in making this sort of extrapolation, it provides the basis for useful hypotheses regarding the pathogenesis of various craniofacial malformations (see also Johnston,[8]). For instance, in otocephaly, which occurs frequently in experimental animals and man (see Fig. 3–8), it seems that in the most severe form of condition all the neural crest derivatives are absent as well as the organs supported by crest tissues. Both in man and in the guinea pig, where it has been extensively studied (Wright and Wagner,[25] the condition is found with varying degrees from complete absence (Fig. 3–8a) to progressively less severe malformations, finally involving only the mandibular arch (Fig. 3–8B). Since the components most often affected are those derived from crest cells which have to undergo the most extensive migration, we have postulated that these defects may result from problems of neural crest cell migration.

Another condition which may be at least partially explained by the involvement of neural crest in its development is familial facial osteodysplasia (Anderson, et al.[1]). In this condition, the bone presumed to be derived from neural crest is extremely hypoplastic. The condition is thought to result from a recessive gene which, in the homozygons condition, could conceivably cause defective terminal induction of bone from neural crest cells (see Fig. 3–12).

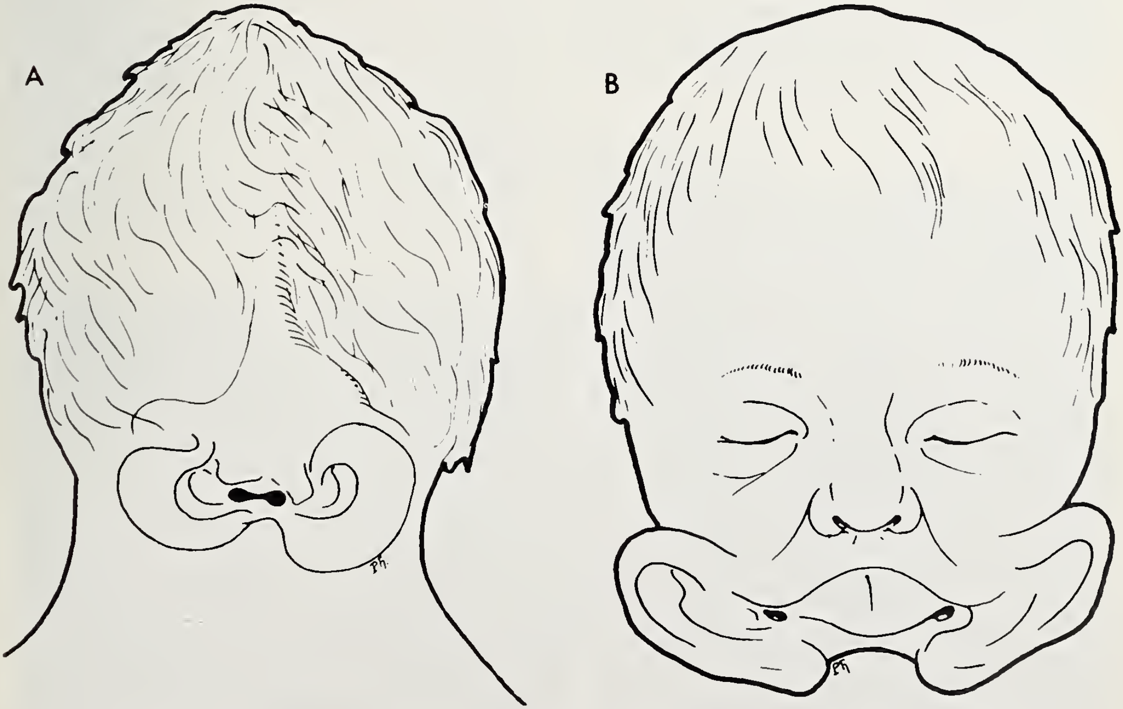

Figure 3–8. Complete (A) and moderate (B) cases of human otocephaly (from Duhamel[4]).

NEURAL DERIVATIVES

The cranial neural crest cells are now known to be able to form sensory and autonomic neurons and also receptor and supporting cells.

Neurons

The use of the quail chromatin marker has permitted further elucidation of the origins of sensory and autonomic neurons (see also Johnston and Hazelton[9]). By using this marker system we have determined the distributions of crest and placodal neurons within the trigeminal ganglion up to prehatching stages. While the significance of the dual origin of neurons in this ganglion is not clear, additional studies on the problem have been initiated with Dr. Peach (see Chapter 3, this Symposium).

Little new information has been added regarding the autonomic ganglia. However, use of the new cell marker has confirmed the vagal crest origins of the parasympathetic ganglia innervating the viscera (see also LeDouarin and Teillet[14]).

Receptors

It has recently been shown that the neural crest participates in the formation of a number of receptors. The organ of Herbst has been extensively studied by Saxod[21,22]) in the duck embryo and he was able to demonstrate contributions of quail crest cells to this receptor. Crest cells also contribute to the formation of chemoreceptors in the carotid body (see Fig. 3–9A and 3–11; also LeDouarin, et al.[15], Pearse, et al.[18]) and baroreceptors (see Figs. 3–9b and 3–11). We have also found crest cells accumulating in high concentrations under developing receptor epithelium (gustatory, olfactory and auditory)—see also Chapter 5 in this Symposium, by Munger. We suggest that these cells may have an inductive role or that they may participate directly in the formation of the receptors.

Pigment cells have been found within taste buds of the sheep by Bradley and Mistretta (personal communication). There are two possible alternatives regarding their function. They may merely be melanoblasts which have entered the

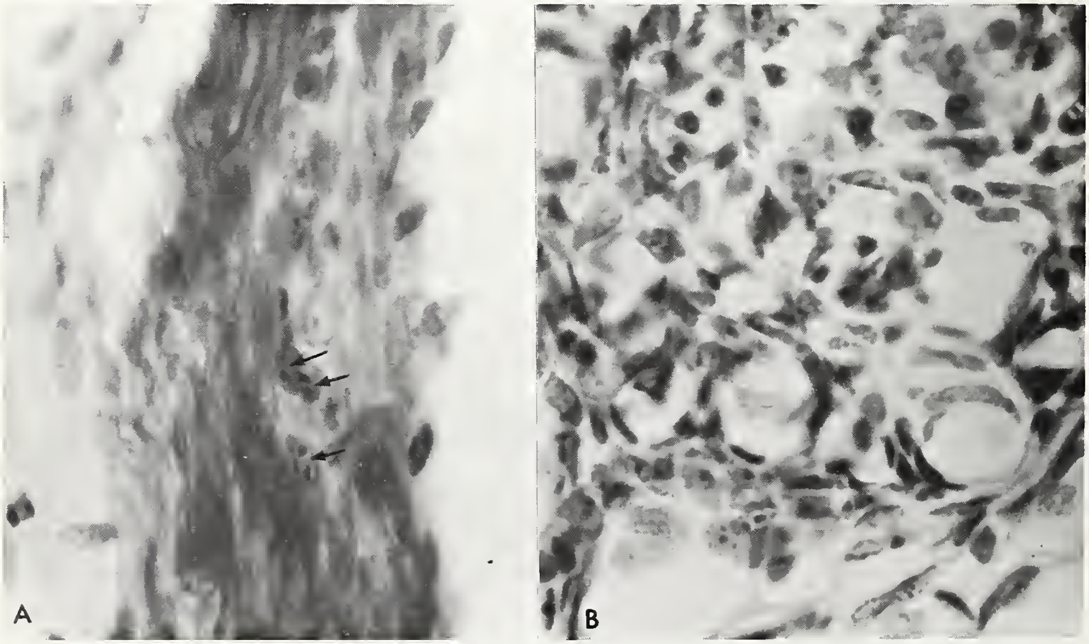

Figure 3–9. A. Cells (arrows) derived from a quail crest graft in the wall of the common carotid. They are presumed to have baroreceptor function. B. Numerous cells derived from quail crest graft in carotid body.

taste bud epithilium. Or they may have another primary function, the formation of melanin being incidental to this function. The latter interpretation would be in agreement with the apparent function of cells of crest origin in the inner ear where they may or may not form melanin, depending upon whether or not the animal being studied is pigmented or non-pigmented. In this case the function of the crest cells appears to be concerned with the regulation of inner ear fluid (D. Lim, personal communication) and this function is not affected by the presence or absence of melanin.

Supporting Cells

The use of the chromatin cell marker has been very helpful in elucidation of origin of the supporting cells found in ganglia and nerves. Since many of these cells differentiate very late and after extensive proliferation, their origins were difficult to determine using the tritiated thymidine label. However, from studies using the chromatin marker, it seems that essentially all the supporting cells are of crest origin. While this had been the earlier interpretation, several workers contributing extensively to the field (e.g. Yntema,[26,27]) have concluded that cells of placodal origin also form supporting satellite cells in the ganglia and that mesodermal cells might also make similar contributions. Our results do not support this conclusion.

ENDOCRINE DERIVATIVES

There has long been general agreement that the adrenalin and noradrenalin producing cells and the adrenal medulla are of neural crest origin. Recent work indicates that a number of other endocrine cells may originate from the neural crest. Based primarily on histochemical characteristics, Pearse[17] postulated that a large group of cells having both endocrine and nonendocrine functions might be derived from the neural crest. This group he termed APUD cells, the letters of which stand for *A*mine *P*recursor *U*ptake and *D*ecarbolyxation. The APUD cell group includes the calcitonin producing cells (C-cells) found in the mammalian thyroid and in the ultimobranchial body of lower vertebrates (e.g. Chan, et al.[2]), the ACTH and MSH producing cells in the anterior pituitary, the insulin and glucagon producing cells in the islets of Langerhans, as well as similar cells found in the carotid body.

Using the quail marker in chick em-

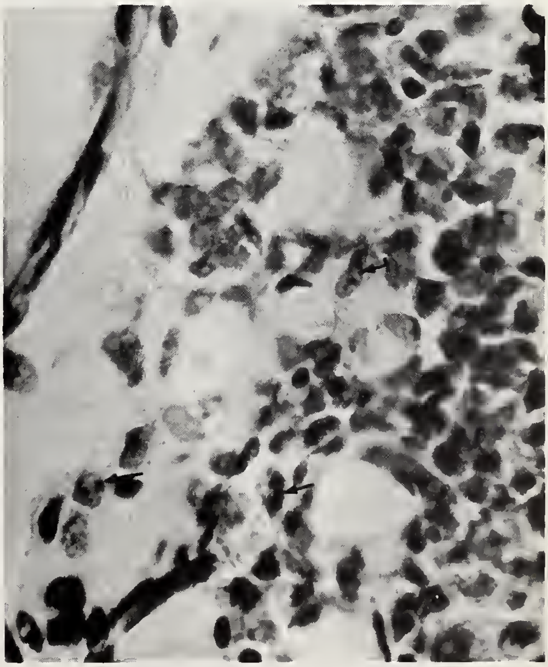

Figure 3–10. Quail crest cells (arrows) in the ultimobranchial body of a chick host. The epithelial (minimum intercellular space) arrangement of these cells is best seen in the contact arrangements of the cell indicated by the crossed arrow.

bryos, LeDouarin, *et al.*,[13] has provided very good evidence that the C-cells are in fact, of crest origin. This observation has been confirmed in our laboratory (see Fig. 3–10). For many years, embryological investigators (e.g. Ishikawa,[7]) have concluded that the ultimobranchial body fuses with the lateral thyroid in mammalian embryos and that this sequence of events probably accounts for the location within the thyroid gland of the C-cells in mammals (see Fig. 3–11). We have found that the mesenchyme cells of the anterior pituitary are of neural crest origin and it is possible that they may form the ACTH and MSH cells. There is some evidence indicating that the cells in the islets of Langerhans producing insulin and glucagon may, however, originate from endodermal epithelial cells (see Pictet, et al.[19] and Pictet and Rutter[20]).

GENERAL COMMENTS

On the basis of studies conducted on experimental embryos (including some preliminary results on mammalian embryos in our laboratory), the cranial neural crest contributes extensively to structures in the oral and pharyngeal regions. From Figure 3–12 it can be seen that a large variety of cell types is derived from this primordium. In some cases there is evidence that the crest cells become progressively differentiated as they migrate past various structures toward their final destination. For example, crest cells migrating past the pharyngeal endoderm seem to become pre-conditioned so that when they come in contact with oral epithelium they interact with this epithelium to produce a tooth primordia with crest cells forming all the structures except the enamal organ (see Weston,[23] for review). Currently, some additional information is available regarding influences directing crest cell migration and differentiation (e.g. Holtfreter,[6] Drews, et al.[3] and Noden, Chapter 2, this Symposium.)

It may be possible to utilize such information concerning the developmental history of tissues to help in understanding the nature of syndromes or other combinations of malformations in the oral and pharyngeal regions. Also, such information may be important in understanding the nature of tumors which appear to originate from crest derivatives. For example, tumors arising from C-cells within the thyroid may produce other hormones such as ACTH (Williams et al.,[24] Gorlin and Mirkin,[5]) that are normally produced by other endocrine cells also presumed to be crest origin.

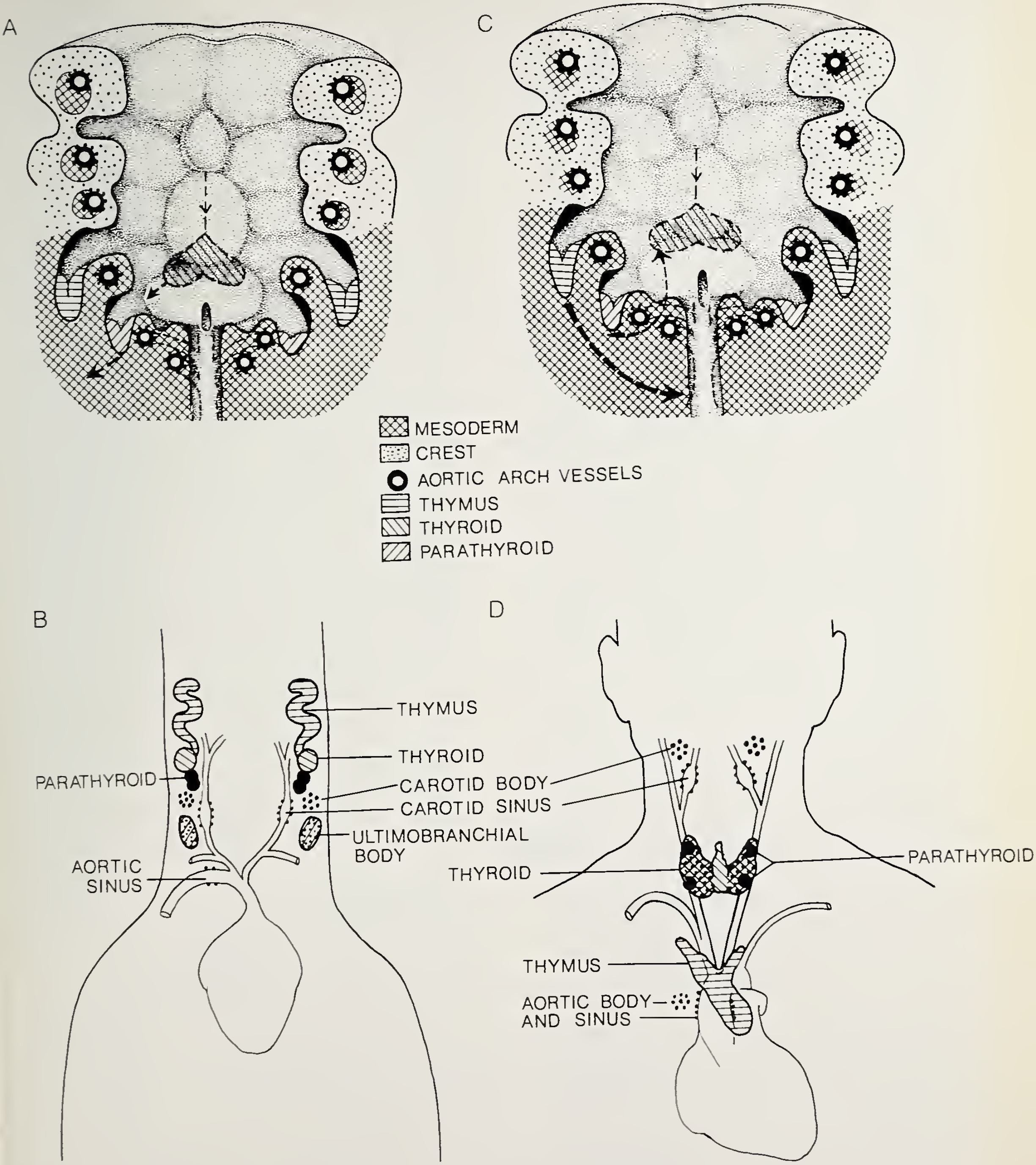

Figure 3–11. Schematic presentation of origins and final positions of pharyngeal glands and vascular receptors in avian species (A, B) and in man (C, D). The dorsal views of the floor of the embryonic pharynx in chick (A) and man (C) show virtually identical positions for the various primordia. Later development leads to movement of the glandular primordia into lateral locations in avian species (B) and into mid-line locations in man (D).

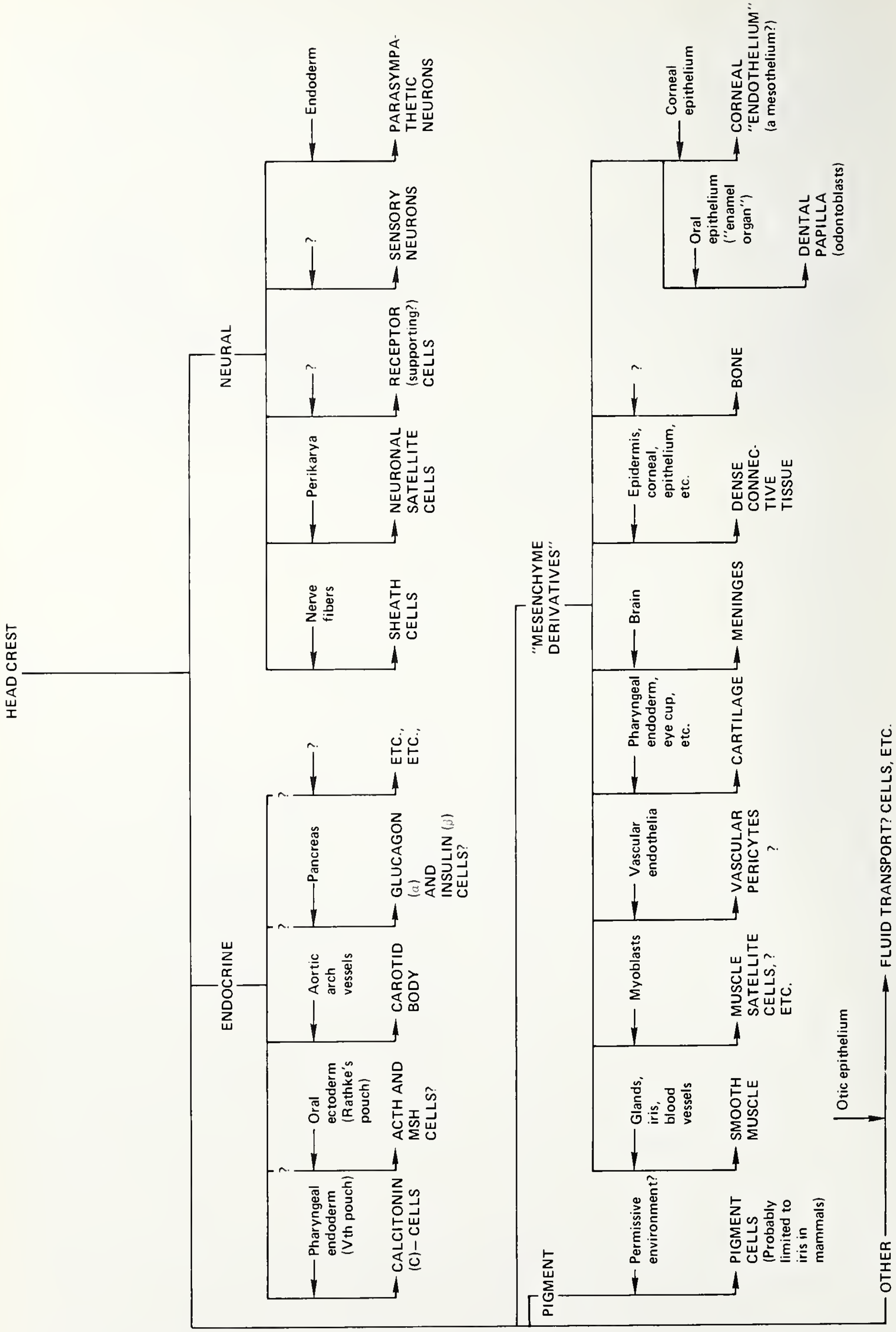
HEAD CREST
NEURAL
Nerve fibers
SHEATH CELLS
Perikarya
NEURONAL SATELLITE CELLS
?
RECEPTOR (supporting?) CELLS
?
SENSORY NEURONS
Endoderm
PARASYMPATHETIC NEURONS
ENDOCRINE
Pharyngeal endoderm (Vth pouch)
CALCITONIN (C)–CELLS
?
Oral ectoderm (Rathke's pouch)
ACTH AND MSH CELLS?
Aortic arch vessels
CAROTID BODY
?
Pancreas
GLUCAGON (α) AND INSULIN (β) CELLS?
?
?
ETC., ETC.,
"MESENCHYME DERIVATIVES"
Glands, iris, blood vessels
SMOOTH MUSCLE
Myoblasts
MUSCLE SATELLITE CELLS, ? ETC.
Vascular endothelia
VASCULAR PERICYTES ?
Pharyngeal endoderm, eye cup, etc.
CARTILAGE
Brain
MENINGES
Epidermis, corneal, epithelium, etc.
DENSE CONNECTIVE TISSUE
?
BONE
Oral epithelium ("enamel organ")
DENTAL PAPILLA (odontoblasts)
Corneal epithelium
CORNEAL "ENDOTHELIUM" (a mesothelium?)
PIGMENT
Permissive environment?
PIGMENT CELLS (Probably limited to iris in mammals)
OTHER
Otic epithelium
FLUID TRANSPORT? CELLS, ETC.

Figure 3–12. Flow chart indicating major groupings of head crest derivatives. The inductive or presumed inductive influences of various tissues on the crest cells are designated by arrows.

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Chapter 4

DEVELOPMENT OF THE TRIGEMINAL GANGLION

Roy Peach

This report* ** describes fine structural features of the trigeminal ganglion in the adult, in the mouse embryo and in mice with a genetically determined peripheral neuropathy which is first apparent shortly after birth. Such fine structural studies necessarily must be done on experimental animals. However, in providing information about structural organization of the mature and developing trigeminal system at the near molecular level, they are directly relevant to the topic of this symposium.

ADULT TRIGEMINAL GANGLION

The trigeminal ganglion contains the cell bodies of sensory exteroceptive and possibly of some proprioceptive neurons, although the majority of proprioceptive fibres have their cell bodies located in the central nervous system.[25,34] The neurons of the trigeminal ganglion are pseudounipolar cells. Functionally the peripheral process is a dendrite and the central process an axon, but structurally the two are similar and will be referred to as processes or axons. Synapses have not been observed in the trigeminal ganglion.

Within the ganglion, groups of neuronal cell bodies lie dispersed among numerous myelinated and nonmyelinated nerve fibres.[8,12,13,51] Several investigations have dealt with the functional and somatotopic distributions of these cell bodies in various species using both neurophysiological and anatomical methods.[3,9,30,37] However such observations have not been correlated with variations in fine structure. Electron microscope studies have concentrated on describing the structure of the individual neurons and supporting cells. Such studies have been made on the human,[4,39] the rat,[12,13,35,51] the guinea pig,[39,40] the rabbit,[39,40] the cat and monkey [39,40,53] ganglia. Other sensory ganglia have also been described at the electron microscope level.[10,24,56,57] Significant species variation has not been reported.

A noteworthy feature of the adult trigeminal ganglion is the apparent presence of two types of neuron.[8,12,50,51] The two differ in size and cytoplasmic density. The cytoplasm of the larger, 'light', cell stains less intensely than that of the smaller, 'dark', cell. The two types are distributed throughout the trigeminal ganglion of the rat (Fig. 4–1) and can be demonstrated by techniques for acid phosphatase.[50] With this method, the lighter reaction deposits occur as discrete cytoplasmic granules in the larger neurons. In the smaller neurons more intense deposits are found as larger granules accompanied by an intense and diffuse cytoplasmic reaction (Fig. 4–2).

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**Part of this work was done in collaboration with Dr. W.E. Koch, Anatomy Department and part with Dr. J.S. Hanker, Dental Research Center, Chapel Hill, and I am grateful for their permission to refer to our joint observations.

Such cytoplasmic differences can be demonstrated by a variety of methods and have been described for other sensory ganglia[56,57] as well as for other parts of the nervous system. Numerous explanations have been advanced to account for these variations,[6,7] but some workers have regarded them as artifacts caused by post-mortem trauma to inadequately fixed tissue.[6,7,43] Indeed, light and dark cells were not seen in the trigeminal ganglia of cat and monkey where the uniform cytoplasmic density did not provide a basis for classification of cells into separate types.[53] However a non-artifactual origin for the two types, as seen at the histochemical and fine structural levels in the trigeminal ganglion of the rat, was indicated by the use of prolonged fixation times and careful handling procedures.[50,51]

Similar fine structural differences are observed among the trigeminal neurons of the adult mouse. The smaller 'dark' cell has a greater concentration of organelles, particularly ribosomes and rough surfaced endoplasmic reticulum

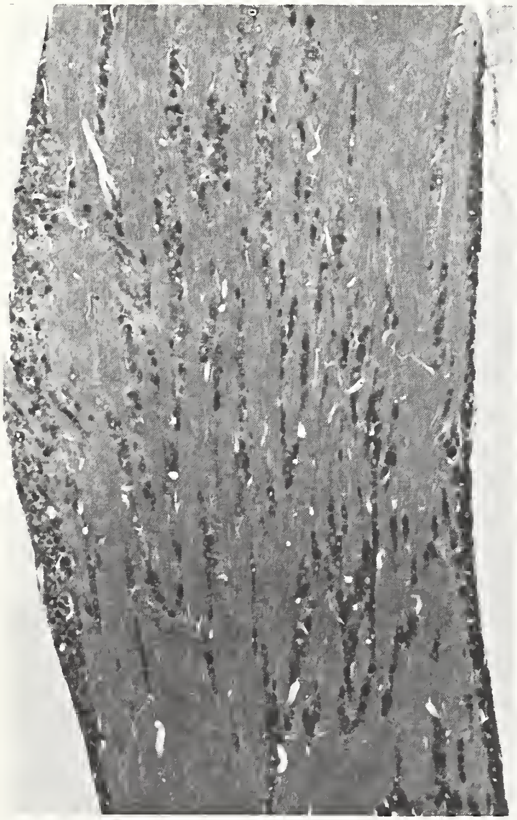

Figure 4–1. Low magnification illustrating distribution of 'light' and 'dark' neurons in rat trigeminal ganglion as demonstrated by technique for acid phosphatase. X 40.

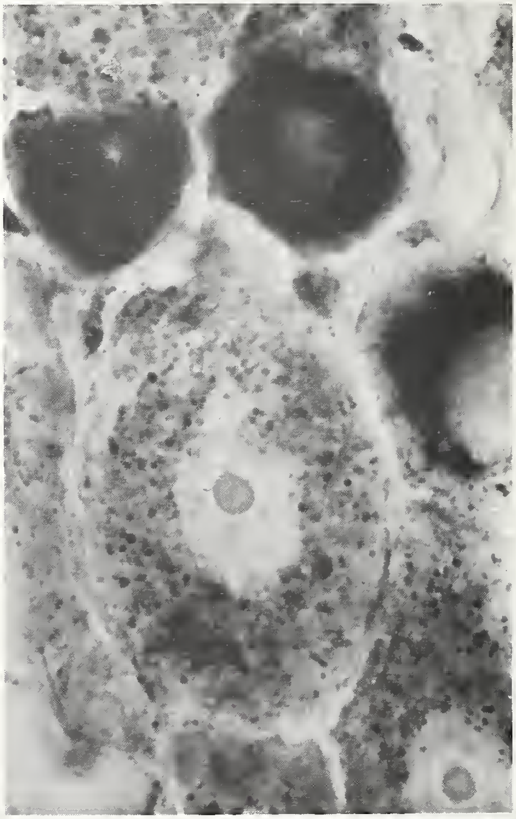

Figure 4–2. Higher magnification showing nature of reaction product. Granular reaction is seen in the large neuron, a diffuse and more intense cytoplasmic reaction in the small neurons. X 1,500.

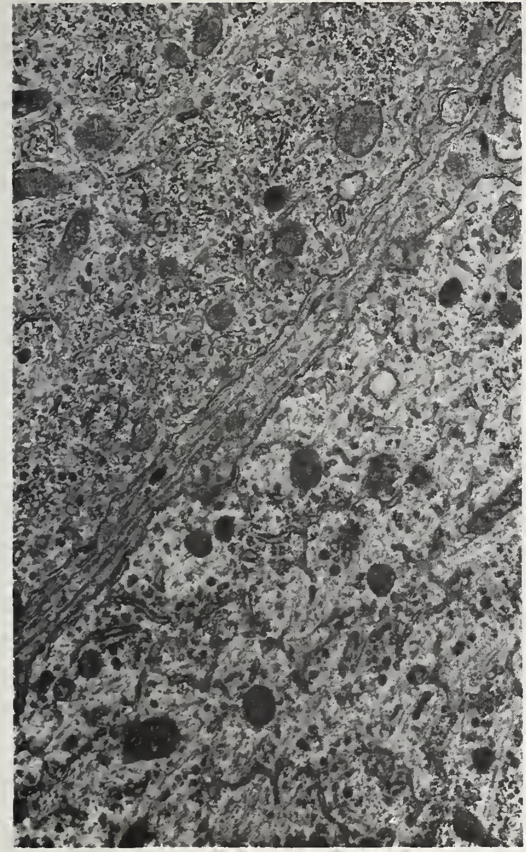

Figure 4–3. Electron micrograph illustrating parts of two adjacent neurons in trigeminal ganglion of adult mouse. The lower cell is a 'dark' cell; the upper cell, a 'light' cell. Each has a satellite cell covering. X 14,000.

(Fig. 4–3). In the light cell the ribosomes have a characteristic clumped appearance as discrete Nissl granules, and the intervening cytoplasm is occupied by numerous neurofilaments (Fig. 4–3). The light cell possesses a simpler Golgi apparatus (Fig. 4–4) than the dark cell (Fig. 4–5). Such differences might account for the acid phosphatase distribution observed with the light microscope. Lysosomal bodies themselves are observed in both types of cell and their distribution does not readily correlate with that for acid phosphatase observed at the light microscope level. The full significance of the cytoplasmic differences remains uncertain. The numerous theories already advanced[7] caution against excessive speculation. Each type of neuron is considered to possess its own characteristic Nissl pattern and this may, in some way, reflect its usual metabolic state.[52] The differences may represent extremes of metabolic activity. An increase in the number of neurofilaments has been associated with enhanced metabolic activity.[1,32] On the other hand the differences might reflect the dual embryological origins of the trigeminal ganglion from neural crest and placodal ectoderm.[28] However light and dark cells are also reported in spinal ganglia[56,57] which are probably derived solely from neural crest.[59] A 'third' type of neuron is present in small numbers in the trigeminal ganglion of the adult mouse. This cell has a narrow rim of cytoplasm and few organelles, but it is regarded as a neuron because of its satellite cell covering.

Satellite cells form a continuous covering over the cell body of each neuron. Their outer contours are relatively smooth but their inner surfaces, adjacent to the neuron, form many complex infoldings (Fig. 4–3). The preservation of these complexities is particularly sensitive to fixation conditions.[49] Satellite cell coverings are continuous with those formed by the Schwann cells which surround the neuronal process. This process follows a spiral course corresponding to Cajal's initial glomerulus.[5] It seems particularly tortuous for some neurons in the mouse trigeminal ganglion where several profiles may be apparent in the plane of section (Fig. 4–6). Upon leaving the vicinity of the cell body, the process bifurcates to give rise to a central branch and a peripheral branch. The fine structure of these has not been described in the trigeminal ganglion. However at the bifurcation in

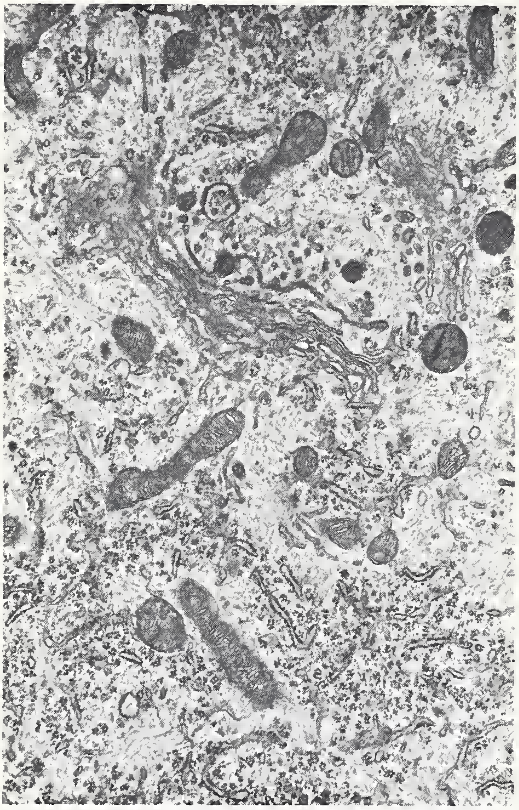

Figure 4–4. Relatively simple Golgi zone from 'light' neuron. Compare with next figure. Adult mouse. X 15,000.

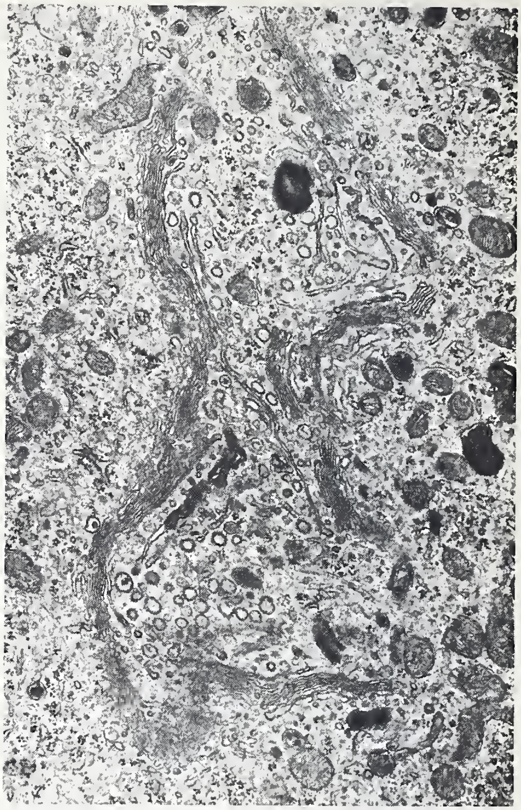

Figure 4–5. Complex Golgi zone from 'dark' neuron. Adult mouse. X 15,000.

the dorsal root ganglia of the cat, myelinated fibres give rise to central and peripheral branches of equal diameter; non-myelinated fibres have a much thicker peripheral process as compared to their central branch.[20]

In addition to neurons the adult ganglion contains connective tissue, fibroblasts, mast cells [36] and blood vessels. The endothelial linings of the latter show microvilli, a feature not widely found in the vascular system.[18]

The trigeminal neurons of one stock of mice used for the present series of observations contain an unusual cytoplasmic inclusion. This consists of a dense particle, surrounded by a smooth surfaced membrane (Fig. 4–7). The membrane encloses a volume approximately 180–200 nm. in diameter and is sometimes continuous with the membranes of the rough-surfaced endoplasmic reticulum. The dense particle has a diame-

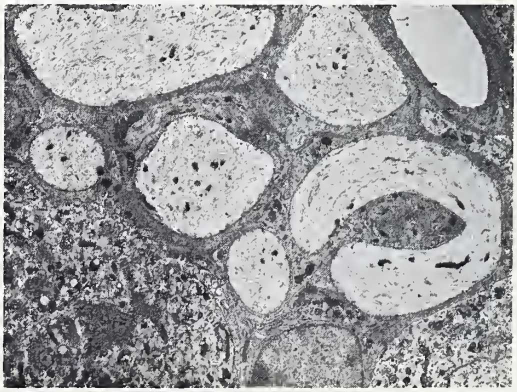

Figure 4–6. Several profiles of initial neuronal process indicative of its tortuous course. Adult mouse. X 2,500.

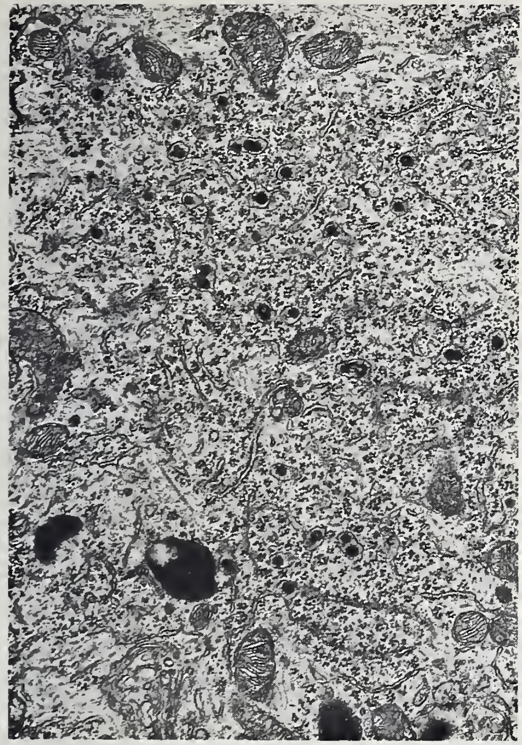

Figure 4–7. Small, dense, viral like, cytoplasmic inclusions. Adult mouse. X 16,000.

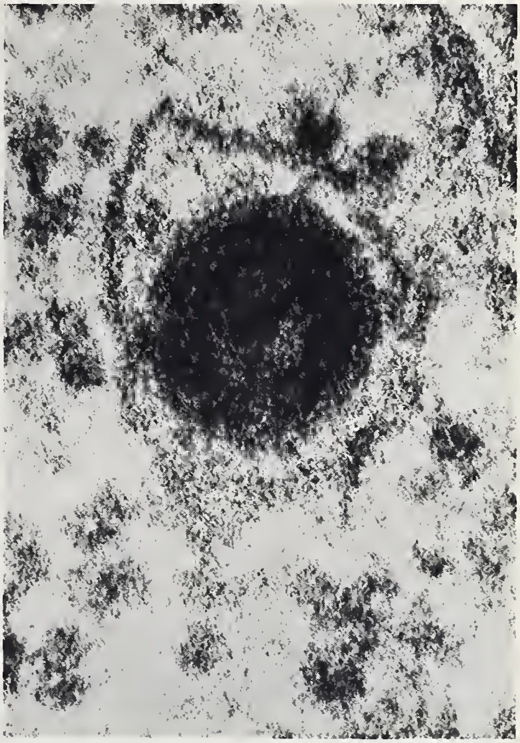

Figure 4–8. One inclusion at higher magnification. Three lamellae can be perceived in the dense material which also shows indications of a substructure. Adult mouse. X 300,000.

ter of approximately 100 nm. and sometimes has an electron lucent center, diameter approximately 40 nm. The dense material around this central zone shows indications of substructure and membranous forms. Up to three concentric lamellae can be perceived but these are not adequately resolved by the methods employed (Fig. 4–8).

The significance of this inclusion is not known; however, indirect evidence suggests that it may be of viral origin. Similar inclusions have not been described in trigeminal ganglia of other species [4,12,35,39,40,51,53] nor could the inclusion be found in trigeminal ganglia from another separately housed stock of mice. However in the present stock the inclusion has been found consistently over a two year period. The animals however appear clinically normal. Thus any viral infection would appear to be in a covert form.

One possibility that merits consideration is whether the inclusion bears any relation to the herpes group of viruses. Many years ago the trigeminal ganglion in man was incriminated as the location of herpes simplex virus during the latent period of infection.[19] More recently direct evidence from experimental animals[41,55] and human autopsy material[2] has supported this view.

The dimensions of the inclusion body are not incompatible with those of viruses of the herpes group[17,38,42,54,60] although there are differences. In none of the cases cited above[2,41,55] was the virus detectable by morphological means during the latent period. Thus its form in this state is not known. There are many possible identities for the inclusion body such as other viruses or a product synthesized by the cell. It obviously merits further investigation. It can be noted that the inclusion is also observed in very small numbers in the embryonic ganglion.

DEVELOPING GANGLION

There have been several fine structural studies of developing sensory ganglia [44-48,56,57] but none have dealt with the trigeminal which has, as one distinctive feature, its dual embryological origins from neural crest cells and placodal ectoderm cells.[21,27,28,59] Initial studies in the mouse have been directed towards determining the stages of development of fine structural organization and cell associations. Ganglia have been examined from mouse embryos of ages 10, 12, 15 and 19 days and from postnatal animals of ages 1, 5, 10 and 20 days.

The trigeminal ganglia of the chick and probably other vertebrates receive contributions from neural crest and placodal ectoderm over an extended time period. Considerable intermingl-

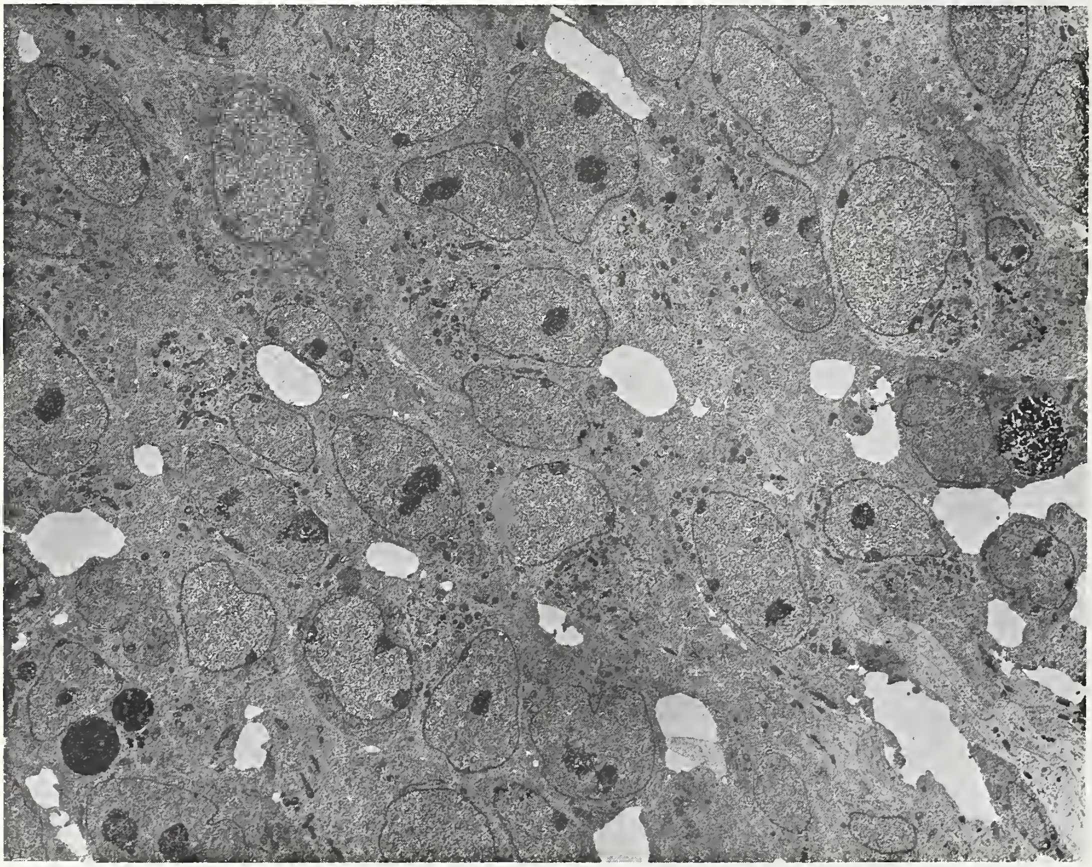

Figure 4–9. Variations in cell shape, nuclear and cytoplasmic densities. Dense cytoplasmic granules in two cells are indicative of cell degeneration (see Figure 4–17). 10 day mouse embryo. X 2,000.

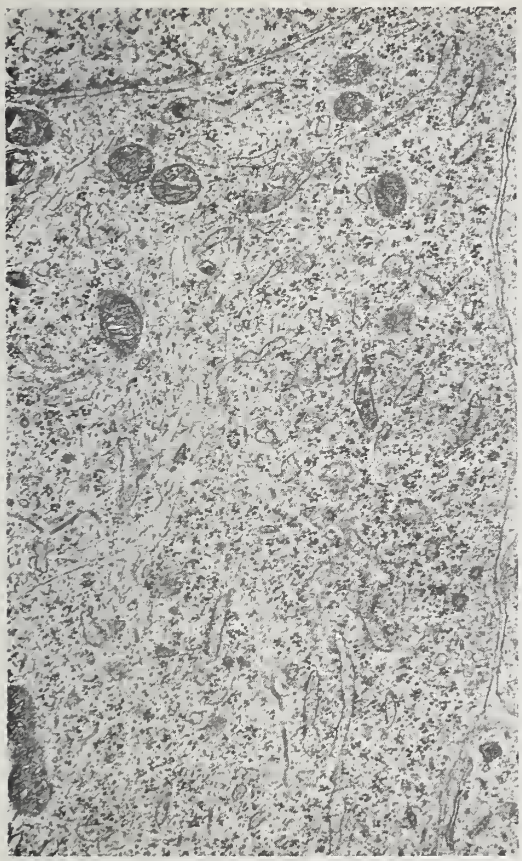

Figure 4–10. Part of long, relatively undifferentiated cell. Cytoplasm contains filaments, free ribosomes, small amounts of rough-surfaced endoplasmic reticulum. 10 day mouse embryo. X 20,000.

ing of the two groups occurs.[28] The cells of the 10-day embryonic mouse trigeminal ganglion form a fairly compact mass and are attached to the developing brain by numerous nerve processes. Serial light microscope sections do not reveal significant correlation between spatial localizations of cells within the ganglion and morphological appearance. The cells show variations in morphology from rounded to elongated forms and also differences in nuclear and cytoplasmic densities (Fig. 4–9). At the fine structural level, long, relatively undifferentiated cells contain numerous free ribosomes and sparse amounts of rough-surfaced endoplasmic reticulum (Fig. 4–10). Fine cytoplasmic filaments are also present but this is not necessarily indicative that these cells are neuroblasts.[33] Some may develop into supporting cells. Other cells appear in rounder form, possessing a more highly organized cytoplasm, including a Golgi zone, bundles of neurofilaments, and more rough-surfaced endoplasmic reticulum (Fig. 4–11). Occasionally, continuity of the rough-surfaced endoplasmic reticulum with the outer nuclear envelope is observed and this may be indicative of its mode of formation. Alternative methods of formation are possible and these have been summarized by Pannese.[47] Tennyson has related the development of fine structural components to the histogenesis of the neuroblasts in the dorsal root ganglia of the rabbit.[56,57] The stages she described were: 1) undifferentiated neuroblasts; 2) spindle and bell-shaped neuroblasts; 3) immature unipolar neurons. The 10-day mouse trigeminal appears to contain cells corresponding to at least stages 1 and 2.

By the twelfth day, neurons possess a richer development of cell organelles, with more rough-surfaced endoplasmic reticulum, numerous small vesicles and elaborate Golgi zones (Fig. 4–12). This fine structural appearance seems to be retained throughout the remaining embryonic stages. Even in the 19-day-old embryos, many neurons have an immature appearance with eccentric nuclei. The rough-surfaced endoplasmic reticulum remains distributed throughout the cytoplasm without exhibiting the characteristic clumped appearance of the light cell (Fig. 4–13). Light and dark cells are not distinguished. However good fixation has been hardest to achieve in the 15–19 day embryos. Con-

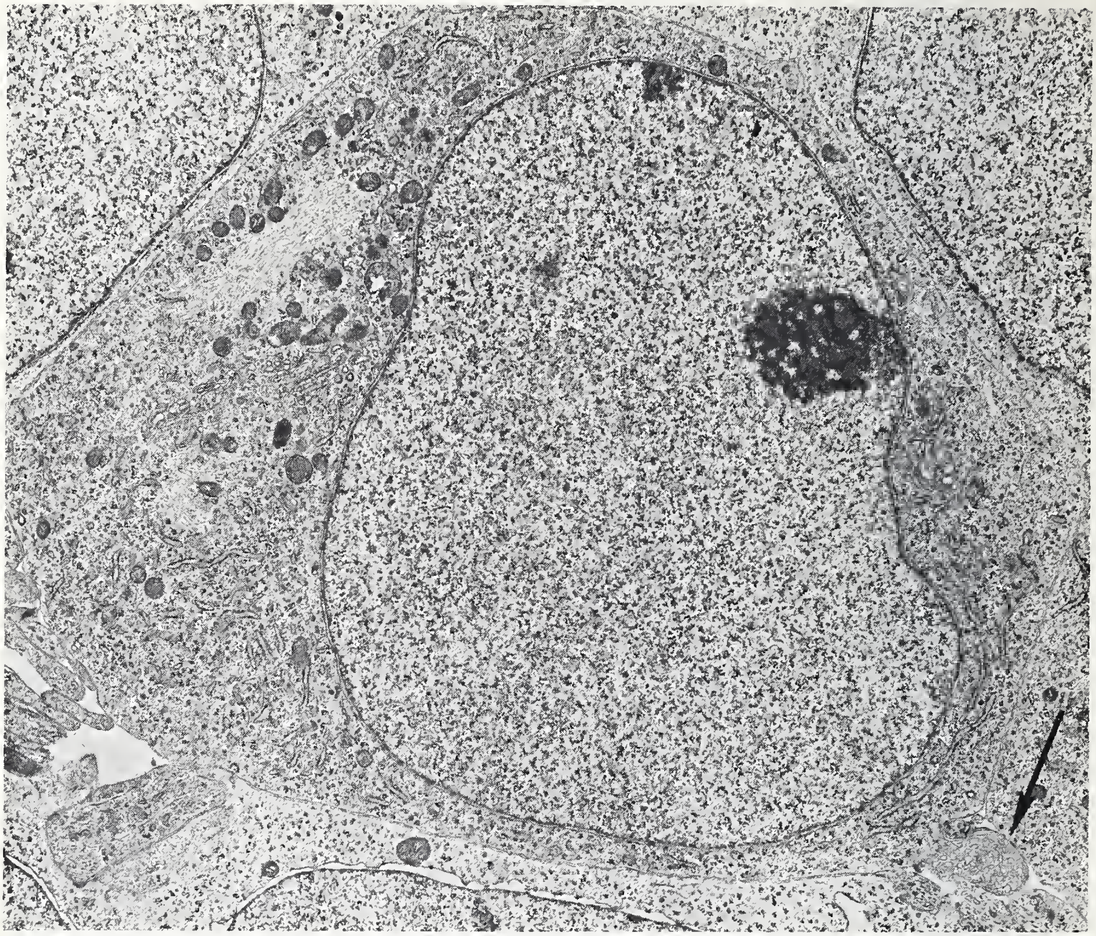

Figure 4–11. Cytoplasmic organization more highly developed in rounded neuron. Bundle of cytoplasmic filaments at upper left. Small protuberance from cell surface contains numerous small vesicles at lower right (arrow). 10 day mouse embryo. X 9,000.

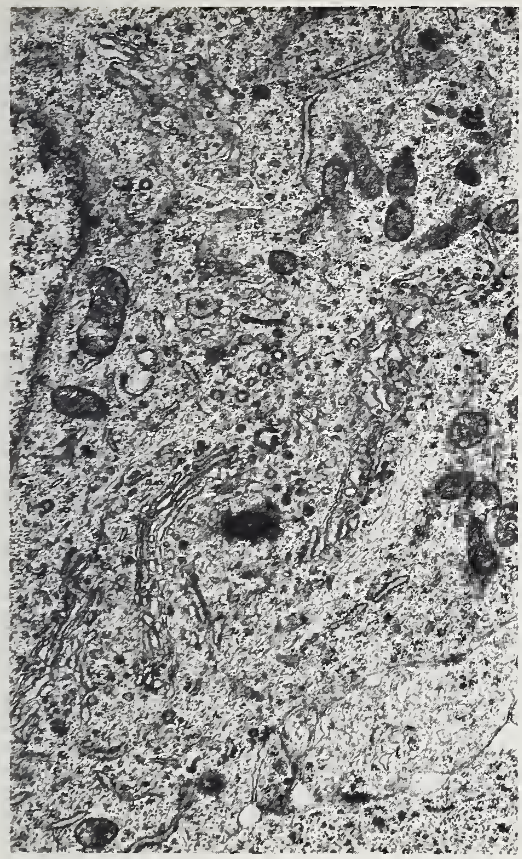

Figure 4–12. Part of one neuron illustrating more highly developed Golgi zone. 12 day mouse embryo. X 13,000.

siderable neuronal maturation takes place post-natally. Light and dark cells are recognized 1 day after birth on the basis of separation of the Nissl substance into discrete clumps by bundles of neurofilaments (Fig. 4–14). A more mature appearance is assumed by days 10–20 (Fig. 4–15).

Thus the recognition of the 'light' and 'dark' cells in terms of their fine structural characteristics has so far been possible only after birth. In the rabbit dorsal root ganglion, however, precursors of light and dark cells were identified in the embryonic stage.[56,57] The light cell had a peripheral accumulation of endoplasmic reticulum whereas the distribution in the dark cell was more uniform. Such distinctions have not yet been made in the embryonic mouse trigeminal ganglion. In the developing spinal ganglion of the chick the gathering of the endoplasmic reticulum into discrete zones as in the 'light' cell was a feature associated with the assumption of a unipolar form by the neuron.[47] In the trigeminal ganglion of the rat differentiation into 'light', mainly large cells and into 'dark', mainly small cells, occurred postnatally as determined by histochemical methods for acid phosphatase and acetylcholinesterase.[29]

Presumptive satellite cells can first be recognized on the 12th day of gestation when their nuclei appear smaller and contain more condensed chromatin than those of the larger neurons. This difference becomes more obvious during later stages. Initially, single satellite cell processes pass between adjacent neurons

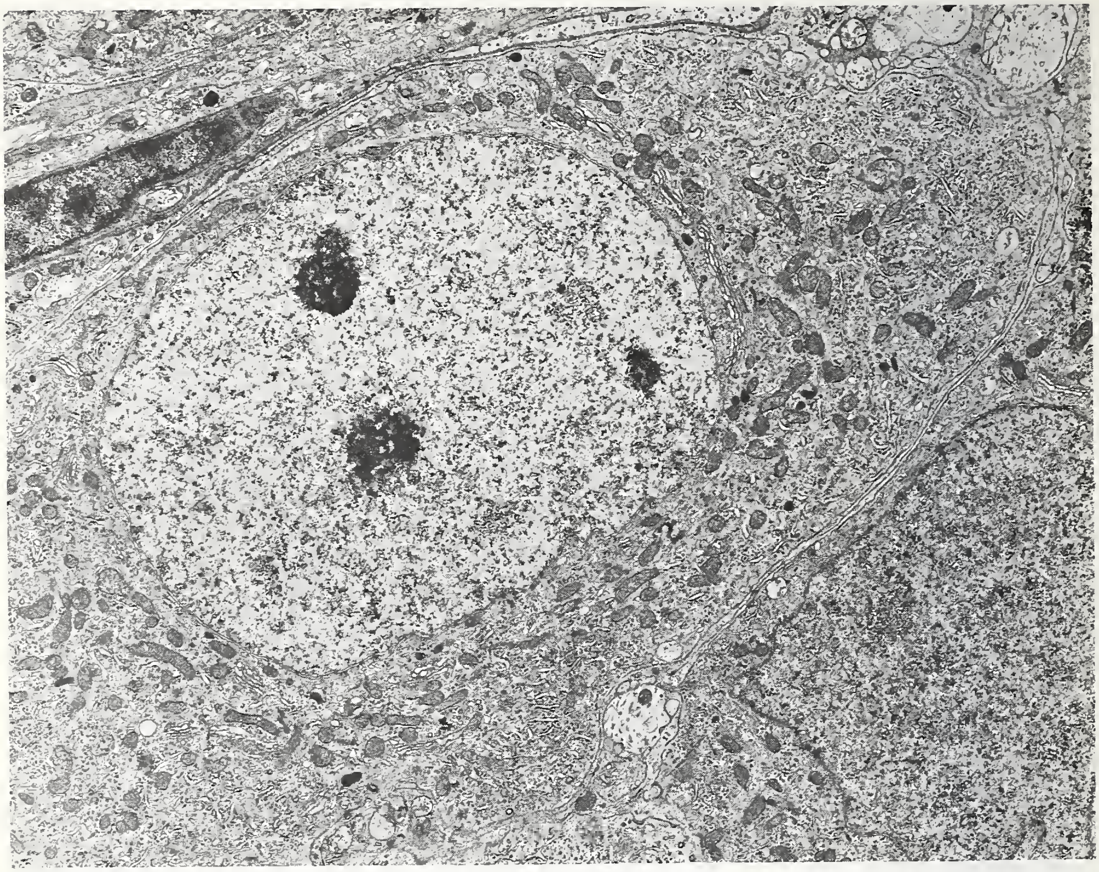

Figure 4–13. Still immature neuron but with more extensive development of cytoplasmic organization. 19 day mouse embryo. X 6,000.

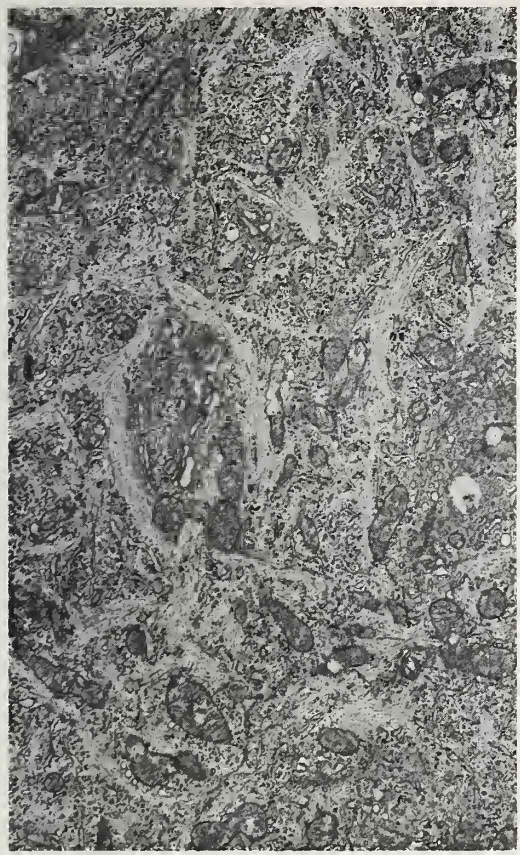

Figure 4–14. Part of 'light' neuron. Numerous neurofilaments pass between clumps of rough-surfaced endoplasmic reticulum. 1 day old mouse. X 17,000.

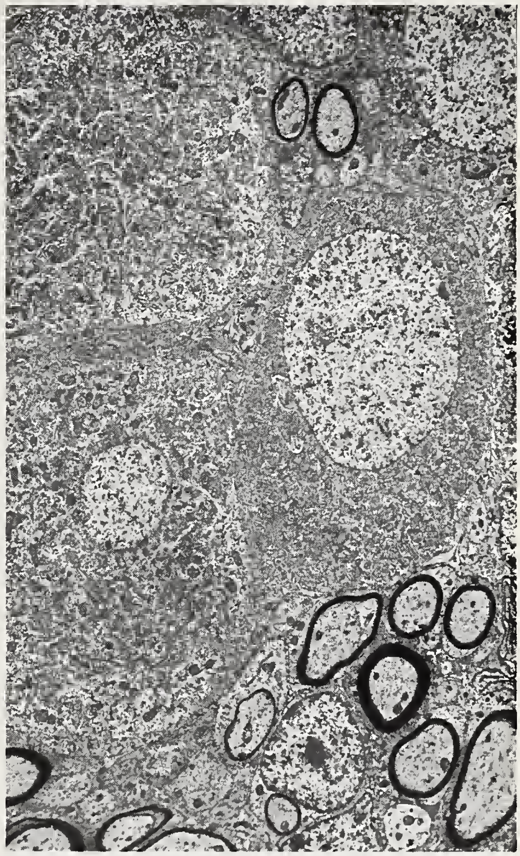

Figure 4–15. One 'dark' neuron and parts of several 'light' neurons. 10 day old mouse. X 2,500.

(Fig. 4–16). Eventually, each neuron has its own satellite cell covering although the covering may not be complete for all neurons even at the 19-day stage. Other satellite cells at this time show complex infoldings on their neuronal aspect: this is a feature typical of the adult.

Temporary cell to cell attachments have been described in early chick neuroblasts [46] but such attachments are found only in small numbers in the mouse trigeminal ganglion. Limited evidence of cell death in the early ganglia is provided by the presence of cytoplasmic inclusions containing remnants of cell organelles (Fig. 4–17). This phenomenon may not be as extensive as reported for the chick spinal ganglia.[22]

In some early cells small, plasma membrane bounded, cytoplasmic protuberances from the neuronal surface are observed. These contain numerous smooth surfaced vesicles and are found on rounded neurons, as well as at the end of long cytoplasmic processes (Fig. 4–11). They have been related to the outgrowth and subsequent extension of dendritic processes from the neuroblast body in the developing cerebellar cortex of rats.[11] However Tennyson [58] has pointed out that the growth cones of the dorsal root neuroblasts of the rabbit are

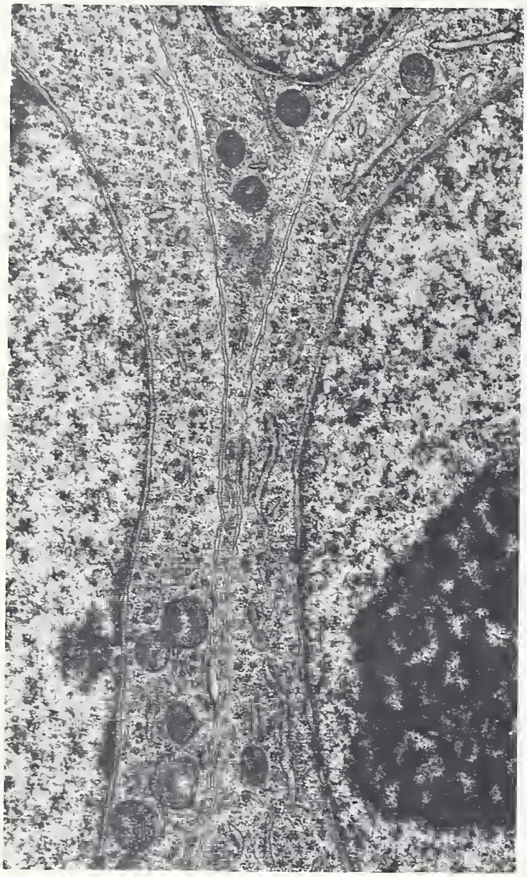

Figure 4–16. Single satellite cell process passes between adjacent neurons. 12 day mouse embryo. X 13,000.

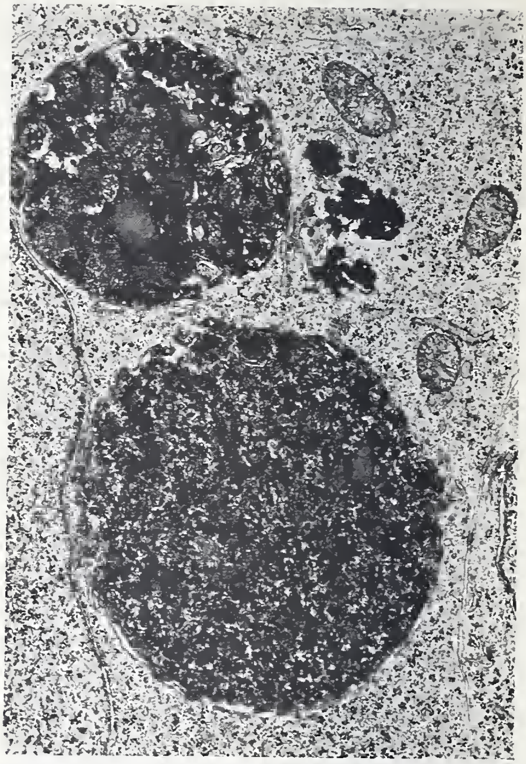

Figure 4–17. Dense cytoplasmic inclusions containing remnants of cell organelles. 10 day mouse embryo. X 15,000.

more elaborate, and the significance of the structures seen in the trigeminal ganglion currently remains uncertain.

Myelinization in the trigeminal system may be another phenomenon related to birth. Nerve processes containing neurofilaments and neurotubules are present in the 10-day embryonic ganglion and already show associations with Schwann cells; the complexity of these associations increases with embryonic age (Fig. 4–18). However myelinization so far has not been observed until after birth. Similarly, myelinization in the trigeminal divisions and palatine nerves of mice was observed by Dixon [14] to be coincident

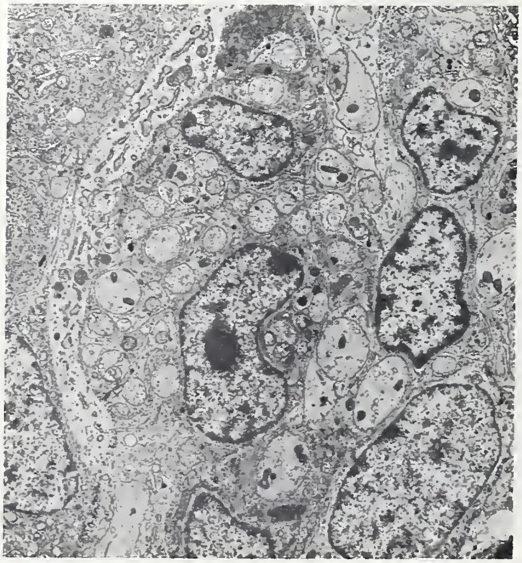

Figure 4–18. Nuclei of Schwann cells in close association with numerous nerve processes. 19 day mouse embryo. X 4,000.

with the time of birth. This author suggested that the emergence of the fetus to the external environment associated with additional demands from the developing peripheral nervous system might necessitate myelin sheath formation to provide more efficient functioning of nerve fibers. This might correlate with a rapid maturation of sensory perception soon after birth.

DYSTONIA MUSCULORUM

The term dystonia musculorum was applied to an hereditary neuromuscular disorder in mice by Duchen, Strich and Falconer.[15,16] The abnormality was described as a severe sensory ataxia characterized by a progressive neuromuscular incoordination, but with no obvious paralysis. The response to painful stimuli was delayed and less brisk than normal.

The disease arose as a spontaneous mutation in a group of randomly bred mice and appeared to be due to a single autosomal recessive gene. Symptoms were not apparent until the sixth day of life and sometimes not until the tenth day. The disorder appeared to affect structures normally formed at birth. The most characteristic pathological abnormality was a degeneration of nerve fibers in the peripheral nerves, in the sensory roots and ganglia of spinal and cranial nerves, and in the spinal cord and brain stem.[15,16]

A stock of these animals is being maintained by Dr. J. S. Hanker in the Dental

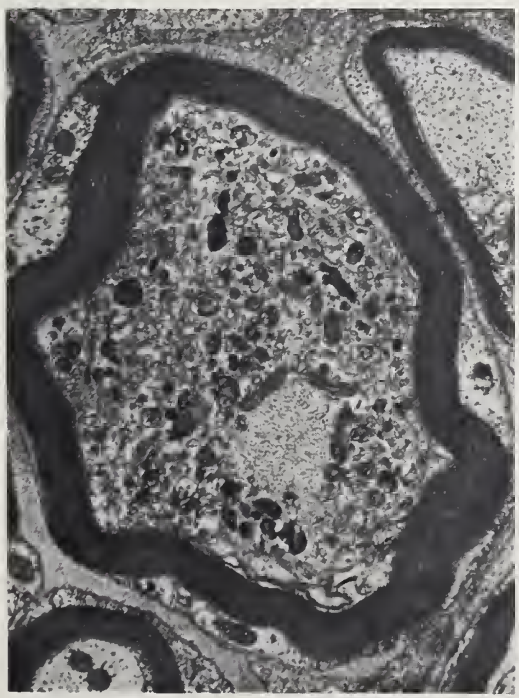

Figure 4–19. Axon packed with organelles. Bundle of neurofilaments lies slightly off axis. Compare with parts of normal axons also in field. Dystonic mouse. X 15,000.

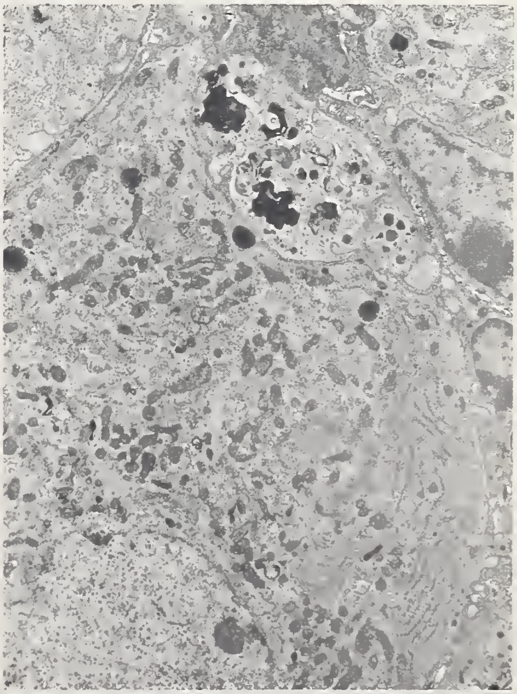

Figure 4–20. Similar accumulation in initial process of trigeminal neuron. Dystonic mouse. X 6,000.

Research Center at Chapel Hill and abnormalities in the development of their trigeminal pathways studied.[23] At the fine structural level the most noticeable changes in the trigeminal ganglion occur in the nerve fibres. Normal axons contain neurofilaments, neurotubules, mitochondria, smooth-surfaced endoplasmic reticulum and vesicles. In abnormal axons, accumulations of dense bodies, mitochondria and structures resembling mitochondrial remnants are found (Fig. 4–19). In some axons the accumulations may occur at the periphery and neurofilaments are characteristically grouped together to occupy an axial position. In others the entire axon may be filled.

That the degenerative process affects neurons whose cell bodies are located in the ganglion is indicated by accumulations in the initial part of the neuronal process (Fig. 4–20). A few cell bodies show peripheral accumulations of Nissl substance; this appearance is associated with chromatolysis following section of peripheral nerves.[e.g.31,37]

Somewhat similar axonal changes are observed in nerve fibres following peripheral injury. The central core of filaments with peripheral accumulations of cell organelle remnants have been described in chronically constricted nerves with uninterrupted fibres.[61] This phenomenon was interpreted as evidence for unimpeded axonal streaming in the axial core with retarded flow in the superficial layers.[61] Thus the defect in dystonia musculorum may be involved in some way with axonal transport. The site of the primary defect, however, remains obscure.

While this presentation was being prepared for publication a fine structural study of dorsal root ganglia, sciatic nerves and sensory nerve roots in mice with dystonia musculorum was published describing similar findings to those reported in the trigeminal ganglion, with the exception of the chromatolytic response.[26] This author points out that the disease might be due to an enzyme deficiency or metabolic abnormality. As the disease appears to be selective for sensory nerve fibres its further study should yield useful information concerning the factors involved in the normal development and maintenance of the trigeminal system.

CONCLUSION

This report has surveyed fine structural features of the adult trigeminal ganglion, its development, and its structure in dystonia musculorum—a genetically determined peripheral neuropathy. The dystonic mice and the unusual 'viral-like' inclusion observed in the adult ganglion merit further investigation. The observations on the developing mouse trigeminal ganglion provide a basis for comparison with studies of the chick trigeminal ganglion. Use of this animal permits the application of the quail/chicken marker techniques described by Dr. Johnston in the previous paper. The significance of the dual embryological origins of the trigeminal ganglion in terms of its fine structural characteristics can then be further explored.

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DISCUSSION

GASSER: The geniculate ganglion in baboon and human embryos develops in that part of the facioacoustic neural crest that is adjacent to the epibranchial placode.[1,2] I have one micrograph of the geniculate ganglion still in contact with the placode. Concerning the trigeminal ganglion, I never observed what one could call a placode adjacent to the trigeminal neural crest.[3] Could Dr. Johnston comment on what the arrangement is in the rat in this region. Is a placode very evident? In the baboon, the mandibular part of the trigeminal neural crest is in contact with the ectoderm but there is no evident placode. Based on this morphology, I would be hesitant to say that a placode contributed to the cells of the trigeminal ganglion.

DR. JOHNSTON: Our studies relating to ganglionic placodes have dealt primarily with transplanted material (crest and placodes) between tritiated thymidine labelled and unlabelled chick embryos[1] or between quail and chick embryos. Also, Dr. Noden has conducted detailed morphological studies on the ganglionic placodes of the chick.[2] Halley's study of cat embryos[1] has been one of the few directed at the ganglionic placodes in mammals. Comparisons of all these studies, including your own, Dr. Gasser, indicates that the morphological development of the ganglionic placodes in each species studied is virtually identical.

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[1] Gasser, R.F.: The development of the facial nerve in man. *Annals of Otology, Rhinol., & Laryngol.*, 76:37–57, 1967.

[2] Gasser, R.F., and Hendrickx, A.G.: The development of the facial nerve in baboon embryos *(Papio sp)*. *J. Comp. Neurol.*, 129:203–218, 1967.

[3] Gasser, R.F., and Hendrickx, A.G.: The development of the trigeminal nerve in baboon embryos *(Papio sp)*. *J. Comp. Neurol.*, 136:159–182, 1969.

[1] Johnston, M.C. and Hazelton, R.D.: Embryonic origins of facial structures related to oral sensory and motor function. In Bosma, J.B. (ed.), *Third Symposium on Oral Sensation and Perception: The Mouth of the Infant*. Chas. C. Thomas, Springfield, Ill., pp. 76–97, 1972.

In the avian embryos which we have studied, the epibranchial placodes for nerves seven, nine and ten are very similar, all three forming in close relation to the pharyngeal endodermal pouches. At the point where the pharyngeal endoderm comes into contact with the surface ectoderm, the ectoderm thickens to form the placode, and it is from that placode that cells migrate into the "neural crest ganglia" of the seventh, ninth, and tenth nerves.

For the fifth nerve ganglion (trigeminal) two "placodes" form, one for the ophthalmic portion and one for the maxillo-mandibular portion. While there is no endodermal contact apparent for these particular placodes, and while the ectoderm there is almost the same thickness as it is in other areas, large numbers of marked cells migrate from these "placodes" into the underlying ganglion.

If I remember your work correctly, you do see what appear to be spurs of cells coming from the "placodes" into the ganglion. In the chick the ophthalmic placodal cells come in single cells or maybe clumps of two or three but never in massive amounts like the placodal cells for the seventh, ninth and tenth nerves. The migration of cells from the maxillo-mandibular placode is somewhat more similar to that of the more posterior epibranchial placodes.

I think they are morphologically quite similar in mammalian embryos and I would expect that there also are cells contributed by the ectodermal placodes to the ganglia, although they haven't been tagged and followed yet.

I wonder if Dr. Noden would like to comment further? He has done a more extensive morphological study.

NODEN: In regard to another aspect of Dr. Gasser's question, following their initial migrations some avian neural crest cells again come into contact with the presumptive placodal epidermis. This is most clearly seen in the mandibulo-maxillary portion of the condensing trigeminal ganglion. However, I have not seen any similar apposition beneath the ophthalmic placodal epidermis.

It would be most interesting to know the developmental significance of these neural crest-presumptive placode interactions. This problem is currently under investigation in my laboratory.

GASSER: One wonders why some crest cells come in contact with the ectoderm. When one considers the functional components of the nerves that these crests will form, there seems to be the best contact with those that have a special visceral motor component. This would include the seventh, ninth and tenth cranial nerves but only the mandibular part of the fifth nerve. Possibly some relationship exists between this motor component and the initial contact with the ectoderm.

JOHNSTON: It is usually thought that the mandibular and ophthalmic portions of the trigeminal ganglia represent the ganglia of the second and third most anterior visceral arches of ancestral vertebrate embryos. Although the "visceral arch" served by the ophthalmic ganglion would then have undergone considerable evolutionary change, the ophthalmic ganglion (i.e., the ophthalmic portion of the trigeminal ganglion) still has combined neural crest and placodal origin, at least in the avian embryos we have studied.

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[1] Halley, G.: The placodal relations of neural crest in the domestic cat. *J. Anat.*, 89, 133–152, 1955.

[2] Noden, D.M.: A radioautographic analysis of the migration of chick cephalic neural crest cells. Ph. D. Thesis, 1972, Washington University, St. Louis.

The migration pattern of cells from the maxillo-mandibular placode is more similar to that of cells from the seventh, ninth, and tenth nerve placodes, with the cells migrating as a more or less solid mass. When the ophthalmic placode cells come in they tend to intermingle with the crest cells considerably more so than they do in the maxillo-mandibular and seventh, ninth, and tenth nerve ganglion. If the ophthalmic portion of the trigeminal is in fact a modified visceral arch ganglion, then the double origin of neurons makes sense, but there are definite morphologic differences between its formation and the formation of the more posterior ganglion. I don't know whether or not it is related to the presence or absence of a motor component.

KENSHALO: I am interested by your suggestion that the neural crest cells may contribute to the formation of receptors in the pharyngeal region. What is the origin of the epithelium of the tongue?

JOHNSTON: The anterior two-thirds in the chick is ectodermal, and the posterior one-third is of endodermal origin.

KENSHALO: Is it the same in mammals?

JOHNSTON: It is thought that in the mammal, as has been proven in the lower vertebrates, the anterior two-thirds has an ectodermal epithelium, and the posterior one-third has an endodermal epithelium.

As I indicated earlier in the main presentation, neural crest cells are found in large numbers under tongue epithelium where taste buds later form. Their distribution is roughly coincident with terminal branches of the IXth nerve in the posterior third of the tongue. Whether they have an inductive function or later form some of the cells in the taste bud is not clear.

Of possible relevance is a study conducted on a specialized receptor in the duck bill called the corpuscle of Herbst.[1,2] When the primordium of the duck bill was transplanted with trigeminal or spinal ganglia from quail embryos, quail cells (presumably of crest origin) were found to form a distinct component of the receptor. Both the initial formation and maintenance of the receptor is dependent on the presence of nerve fibers. It is thought that the quail cells come in with the nerve fibers and may possibly be modified sheath cells. Any role they may play in the induction or maintenance of the receptor is not clear.

Regenerating taste buds might require crest cells, possibly derived from sheath cells. I think the experimental data dealing with taste bud regeneration is consistent with this hypothesis, but there are others here who should comment since they have dealt more directly with that problem.

ZALEWSKI: We have been studying the influence of nerves on taste buds as well as which type of epithelia can form taste buds. In rats the whole surface of

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[1] Saxod, R.: Sur l'origine des différentes catégories cellulaires du corpuscle de Herbst. C. R. Acad. Sci. Ser. D, 273, 89–91, 1971.

[2] Saxod, R.: Rôle du nerf et du territoire cutané dans le développement des corpuscules de Herbst et de Grandry. J. Embryol. exp. Morph. 27, 2, pp. 277–300, 1972.

the tongue seems capable of supporting buds. Taste buds are normally found in the fungiform, foliate, and vallate papillae. However, after reinnervation of the tongue "in situ" or of tongue grafts, some buds appear in filiform papillae and in surface epithelium around the papilla [1,3-5] that is, in tongue epithelium where they are never otherwise found. On the other hand, taste buds do not develop in epithelium of skin when skin grafts are reinnvervated by nerves which normally cause bud regeneration.[4] Epithelia thus differ in their ability to be transformed into taste buds.

Transplantation studies have also demonstrated that sensory nerves in dorsal root ganglia can induce taste bud regeneration.[5] When vagal, nodose or lumbar ganglia were transplanted and combined with tongue grafts, taste buds formed. The gustatory specificity attributed to the neurons of cranial ganglia of the chorda tympani, glossopharyngeal, and vagus nerves must now be extended to neurons in dorsal root ganglia.

I do not know whether neural crest cells contribute to the peripheral interaction which causes taste buds to appear. Any neural crest cells which have migrated to tongue papillae would probably be removed when the papillae are excised. However, if the vallate papilla is excised, regenerated taste buds appear in the healed tongue epithelium.[2] Consequently, I doubt whether neural crest cells, rather than lingual epithelial cells, are transformed by the nerve into taste bud cells.

DR. JOHNSTON: The people working with the corpuscle of Herbst, found that the origin of the nerve or ganglion didn't make any difference. So that would be somewhat similar to the taste buds in Dr. Zalewski's studies.

In birds, taste buds are apparently found only on the posterior third of the tongue, and only the glossopharyngeal nerve is concerned with taste.

STEINER: Dr. Peach, do you have an explanation, in terms of functional differences between the so-called dark and light cells.

PEACH: As I indicated in my presentation, many suggestions have been made to account for the differences between the light and dark cells of the nervous system. Explanations in terms of functional differences are speculative. In the case of the trigeminal ganglion it occurred to us that the dual embryological origins of the ganglion might be a factor and we are currently investigating this possibility. In the case of spinal ganglion cells of the rat, cell size appears to be related to axoplasmic volume.[6] This would not in itself account for the differences in staining characteristics. These may represent different levels of metabolic activity. Although there is the correlation with cell size the light cell may be more mature than the dark cell.

STEINER: My impression is that the differentiation of dark and light cells can

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[1] Zalewski, A.A.: Combined effects of testosterone and motor, sensory, or gustatory nerve reinnervation on the regeneration of taste buds in the rat. *Exp. Neurol.*, 24:285–297, 1969.

[3] Zalewski, A.A.: Regeneration of taste buds after transplantation of tongue and ganglia grafts to the anterior chamber of the eye. *Exp. Neurol.*, 35:519–528, 1972.

[4] Zalewski, A.A.: Trophic function of homografted neurons of Ag-B-histocompatible rats. Transplantation, 14:618–623, 1972.

[5] Zalewski, A.A.: Regeneration of taste buds in tongue grafts after reinnervation by neurons in transplanted lumbar ganglia. *Exp. Neurol.* "in press".

[2] Zalewski, A.A.: Regeneration of taste buds in the lingual epithelium after excision of the vallate papilla. *Exp. Neurol.*, 26:621–629, 1970.

[6] Martinez, A.J. & Friede, R.L.: Changes in the nerve cell bodies during the myelination of their axons. *J. Comp. Neurol.* *138*: 329–338, 1970.

be more due to the age of the cells than to a functional discrimination between them.

PEACH: Yes, this is a possibility.

KAWAMURA: You also described morphological differences in the embryonic ganglion including the terms round and long cells. Are there any differences in the dissemination of these cells?

PEACH: The terms light and dark were restricted to the post-natal animal. The early neuron is a bipolar cell which transforms into a unipolar cell which, apart from its process, has a rounder appearance when seen in the electron microscope.

KAWAMURA: I want to ask which cells whether the small cells or large cells are the more affected in the case of dystonia?

PEACH: So far we have only seen changes in light cells but more thorough examination may reveal changes in the dark cells.

BOSMA: Apropos of Dr. Peach's clinical item concerned with a genetic primary degeneration of the sensory system in a laboratory rodent, notice that his primary sensory disorder is named a muscular dystonia. We should ask how many so-called muscular disorders are primarily sensory abnormalities.

NODEN: You mentioned that the abnormalities in otocephalic guinea pigs penetrate in a graduated, rostro-caudal manner. I would like to know whether the cranial ganglia form when the corresponding visceral arches are reduced or absent? This might provide a clue as to whether or not we are dealing with a mutation expressed in the neural crest population.

Secondly, if the ganglia do form normally they are going to have a reduced peripheral field. According to classical studies on spinal ganglia this should result in hypoplasia both of the sensory neurons and the trigeminal motor nuclei. Has this been looked into?

JOHNSTON: The person who studied it most extensively in animals is Sewell Wright [1] and to my awareness he has not studied the development of ganglia or motor nuclei. Mostly he had conducted gross studies and studies of the skeleton.

STEINER: I would like to ask whether arthrogryposis, a congenital malformation which causes abnormal shape of the lower region of the face and which may also distort joints of the limbs,[1] can be interpreted as a disorder of neural crest migration.

BOSMA: Let us further specify arthrogryposis as a syndrome of embryonal failure of development of the motor unit. The motor unit is of composite origin, including elements derived from the neural tube, neural crest and mesoderm. Whether the primary hypoplasia is in the centrally located motoneuron, in its efferent dendrite or in the primordia of the muscle, the resulting abnormality at birth is the same: severe hypoplasia and immobility of the skeletal musculature which is involved. As a secondary phenomenon, the skeleton associated with this musculature is hypoplastic, and its joints are fixed.[1]

The abnormality may be sparsely disseminated, with expression in single or few muscles. Or it may be widespread, as "arthrogryposis multiplex congenita". Murray and Drachman[2] comment that "mice and humans with deficiency of skeletal musculature during embryonic development showed marked retrusion of the mandible."

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[1] Wright, S., and Wagner, K.: Types of subnormal development of the head from inbred strains of guinea pigs and their bearing on the classification and interpretation of vertebrate monsters. *Am. J. Anat.*, 54:383–448, 1934.

[1] Garfunkel, A., and Steiner, J.E.: Orofacial manifestations in arthrogryposis. *J. of Oral Med.* 26:77–81, 1971.

[1] Drachman, D.B.; Normal development and congenital malformation of joints, *Bulletin of the Rheumatic Diseases*, 19:536–540, 1969.

[2] Murray, P.D.F., and Drachman, D.B.: The role of movement in the development of joints and related structures: the head and neck in the chick embryo. *J. Embryol. Exp. Morph.*, 999–1009, 1969.

Chapter 5

CYTOLOGY AND ULTRASTRUCTURE OF SENSORY RECEPTORS IN THE ADULT AND NEWBORN PRIMATE TONGUE

BRYCE L. MUNGER

The tongue has a unique role in neonatal behavior of all mammalian organisms. Feeding is necessary for survival even in marsupials, such as the opossum, where the level of maturity at birth can only be regarded as embryonic. As described elsewhere in this symposium,[35,36] the human newborn tongue evidences discrete reflexes that can be regarded as sensory-motor reflex arcs involving the cranial nerves.

The present study of monkey lingual sensory nerve endings was initiated in an effort to better understand the growth and differentiation of presumed sensory terminals. A carefully timed series of rhesus monkey embryos and infants were examined by light and electron microscopy and compared with equivalent material taken from 3 adult rhesus monkeys.

MATERIALS AND METHODS

Animals of the following ages have been studied: 3 adults; 4 juveniles aged 5 months, 1 month, 3 days and 0 days (removed by hysterotomy when the cervix had begun to dilate); and 3 embryos ~3 months (6.7 cm. crown-rump [C-R] length), ~4 months (19 cm. C-R length) and 5 months (20 cm. C-R length). The postnatal animals were anesthetized with Nembutal; the thoracic cage opened, and the cardiovascular system flushed with warmed 0.9% saline. The embryonic animals did not need additional anesthesia beyond that given to the mother for the hysterotomy. The perfusion mixture was then switched to a dilute solution of paraformaldehyde-glutaraldehyde. The standard hyperosmolar paraformaldehyde-glutaraldehyde fixative [14] was diluted to 1:7 with 0.1M cacodylate buffer. The resulting final osmolarity was ~340 mOsm. Four percent dextran was added to the final fixative to act as a plasma expander and prevent massive transfer of water from the vascular compartment to the connective tissue compartment.[1] Tissue was then removed from the predetermined

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areas of the oral mucosa and specimens prepared for light and electron microscopy.

For light microscopy, blocks of tissue were removed from the perfused animal and fixed in 10% neutral buffered formalin [15] for 2–3 days. Serial sections of paraffin-embedded tissue were stained with a modified Bielschowsky silver impregnation.[29,32] A section was also routinely stained with hematoxylin and eosin (H & E).

For electron microscopy, small blocks of tissue were removed adjacent to those taken for light microscopy and fixed in full-strength Karnovsky's fixative for 2 hours. Tissues were rinsed with several changes of buffer and left in the refrigerator overnight. Tissues were stained *en bloc* with uranyl acetate-oxalate at pH 7.2 [19] and then osmicated in 1.33% OsO_4 in 0.1M s-collidine buffer at pH 7.2, 4° C. for 1 hour. Tissue blocks were embedded in araldite (Durcupan, Fluka), sectioned on diamond knives, stained with lead hydroxide [18] and examined in an RCA–EMU–IV or Philips–300 electron microscope.

RESULTS

Adult

The tongue of the adult rhesus monkey contains a series of fungiform papillae along the anterior margin in which sensory corpuscles are numerous. Other fungiform papillae along the dorsum of the tongue do not have these collections of corpuscular endings. In this context, I regard the corpuscular endings as those nerve terminals that clearly are demarcated from the connective tissue stroma.[21] The term "encapsulated" will not be used. Some terminals have true capsules defined as perineural epithelium.[33] Most of the receptors in monkey tongue do not have a true capsule, but still by light microscopy are demarcated from the connective tissue compartment.

The density of sensory nerve endings in the fungiform papillae at the anterior margin of the primate tongue can only be appreciated by examining serial sections stained with silver (Fig. 5–1A,B). As one proceeds from the midline laterally, only those papillae at the free margin of the tongue contain numerous sensory terminals. A given papilla might contain 3–5 corpuscular endings. In addition, these papillae have typical taste buds and small diameter presumably gustatory fibers in the vascular stroma of the papillae. These papillae containing numerous corpuscles are confined to a row parallel to the free margin along the dorsum of the tongue.

Two types of corpuscular endings can be recognized by light microscopy in these fungiform papillae. One resembles a Meissner corpuscle (Figs. 5–1A, 5–2A), the other is a loosely coiled ending lacking the tight packing of cellular

Figures 5–1A and 5–1B. These two light micrographs are taken from consecutive silver-stained sections from the tip of adult rhesus monkey tongue. A large fungiform papilla is present to the left and the edge of another is to the right of center. Numerous corpuscular receptors can be seen even at this low magnification. Glomerular (G) and Meissner (M) corpuscles can be identified on the basis of the regularity of the looping of the terminal neurite and the stacks of lamellae that can be seen at higher magnification as in Figures 2A and 2B. (X154).

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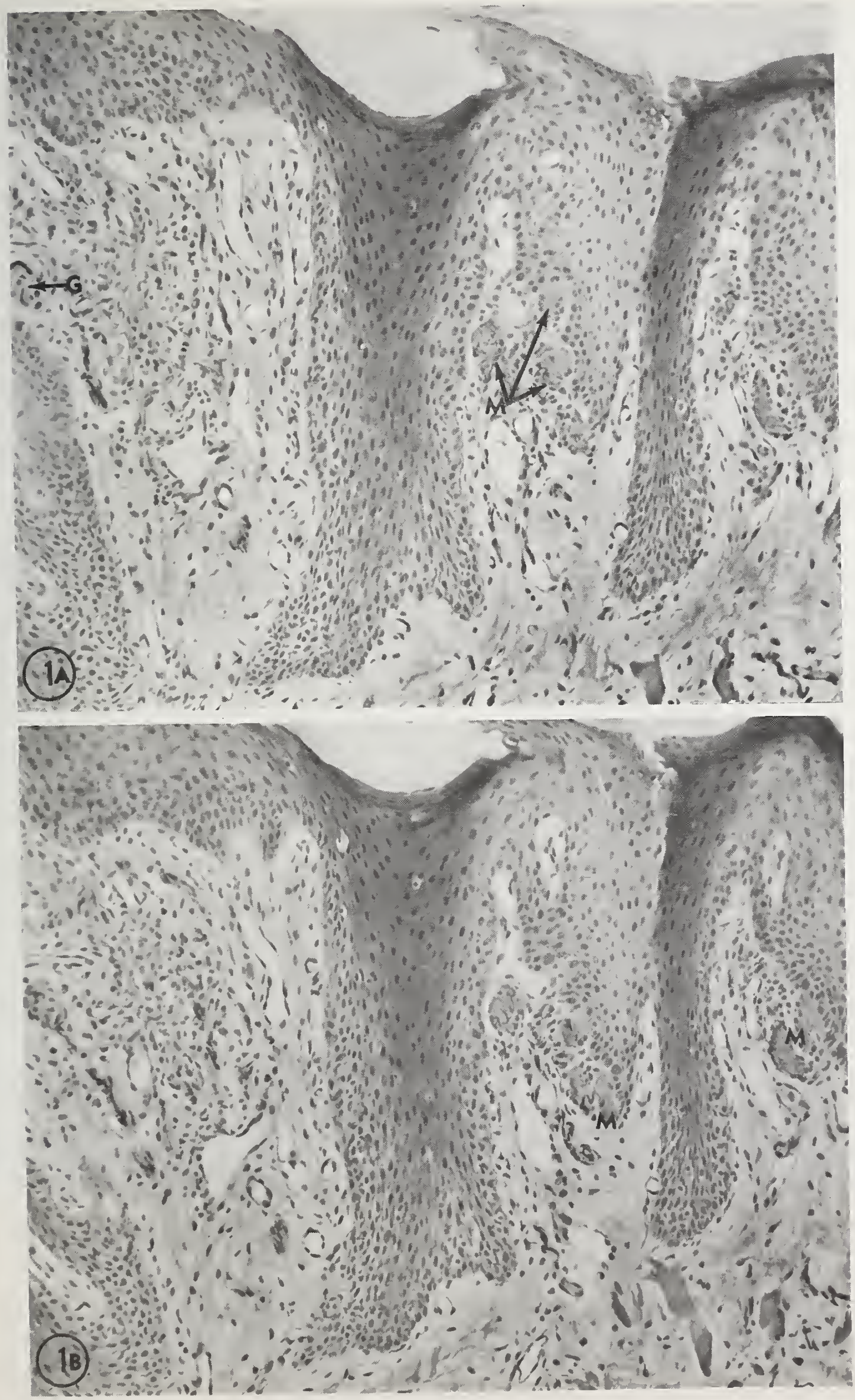
G
M
1A
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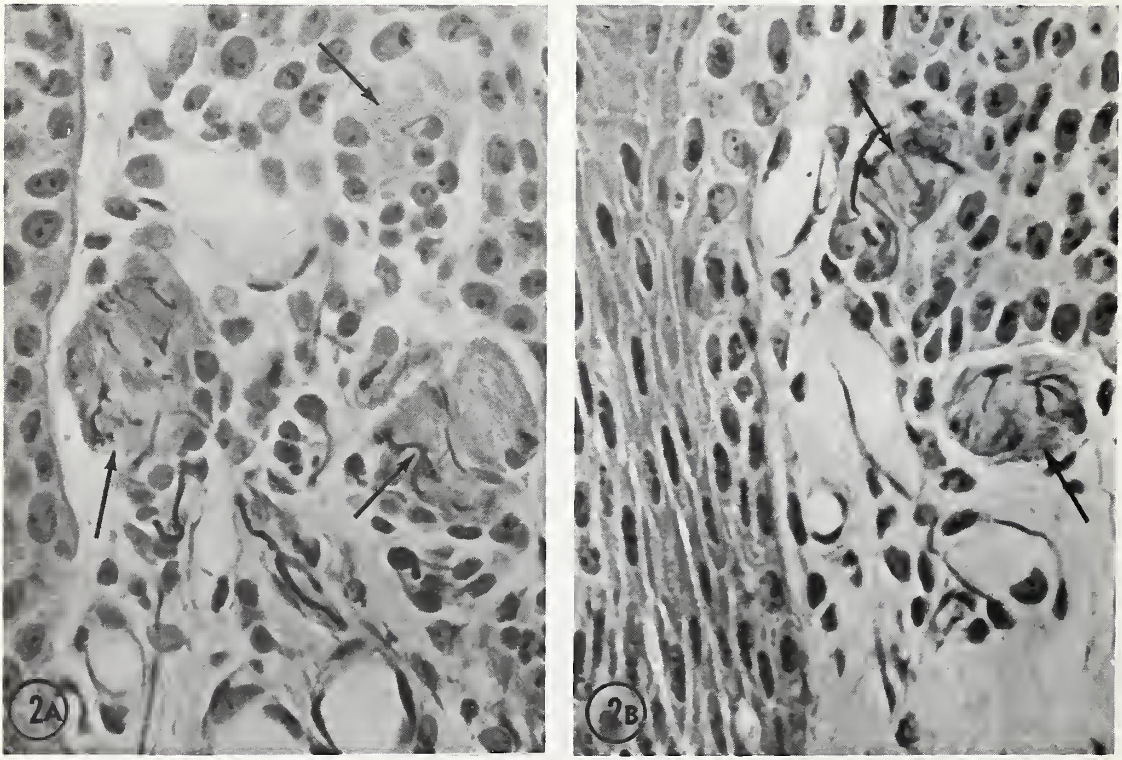
elements characteristic of Meissner corpuscles. These less tightly packed corpuscles will be referred to as glomerular corpuscles (Fig. 5–2B), as suggested by Seto.[30,31] This terminology will be justified in more detail in the discussion.

Meissner corpuscles of the adult monkey tongue closely resembles those encountered in primate digital skin.[2,3] In silver stained preparations, the terminal neurite loops back and forth coursing through successive cellular lamellae seen as faint lamellar profiles (Fig. 2A).

By electron microscopy, the Meissner corpuscles can be easily recognized by the regularity and close packing of cytoplasmic lamellae in the corpuscle (Fig. 5–3). The so-called lamellar cell[21] is a specialized epithelial cell which gives rise to numerous thin sheets of cytoplasm some of which come into intimate contact with the terminal neurite. The cytoplasmic lamellae have numerous pinocytotic vesicles (Figs. 5–4, 5–5, 5–6); in fact, this feature is characteristic of all lamellar cells studied to date including Pacinian corpuscles, Herbst corpuscles, and genital end bulbs where lamellar cells are present.[21] A delicate basal lamina encircles each cytoplasmic lamella and also envelops the cell body of the lamellar cell (Figs. 5–5, 5–6).



Figures 5–2A and 5–2B. Figure 2A is the same field as depicted in Figure 1A with the three arrows. Three Meissner corpuscles (arrows) are present. The upper has only a small portion of blackened neurite, but the lamellae appear as faint striations. The other two corpuscles have the characteristic parallel loops of the terminal neurite. Figure 2B contains portions of two glomerular corpuscles (arrows). Each has irregular convolutions of the terminal neurite. The upper corpuscle in 2A and 2B is very closely applied to the overlying oral mucosa. (X610).

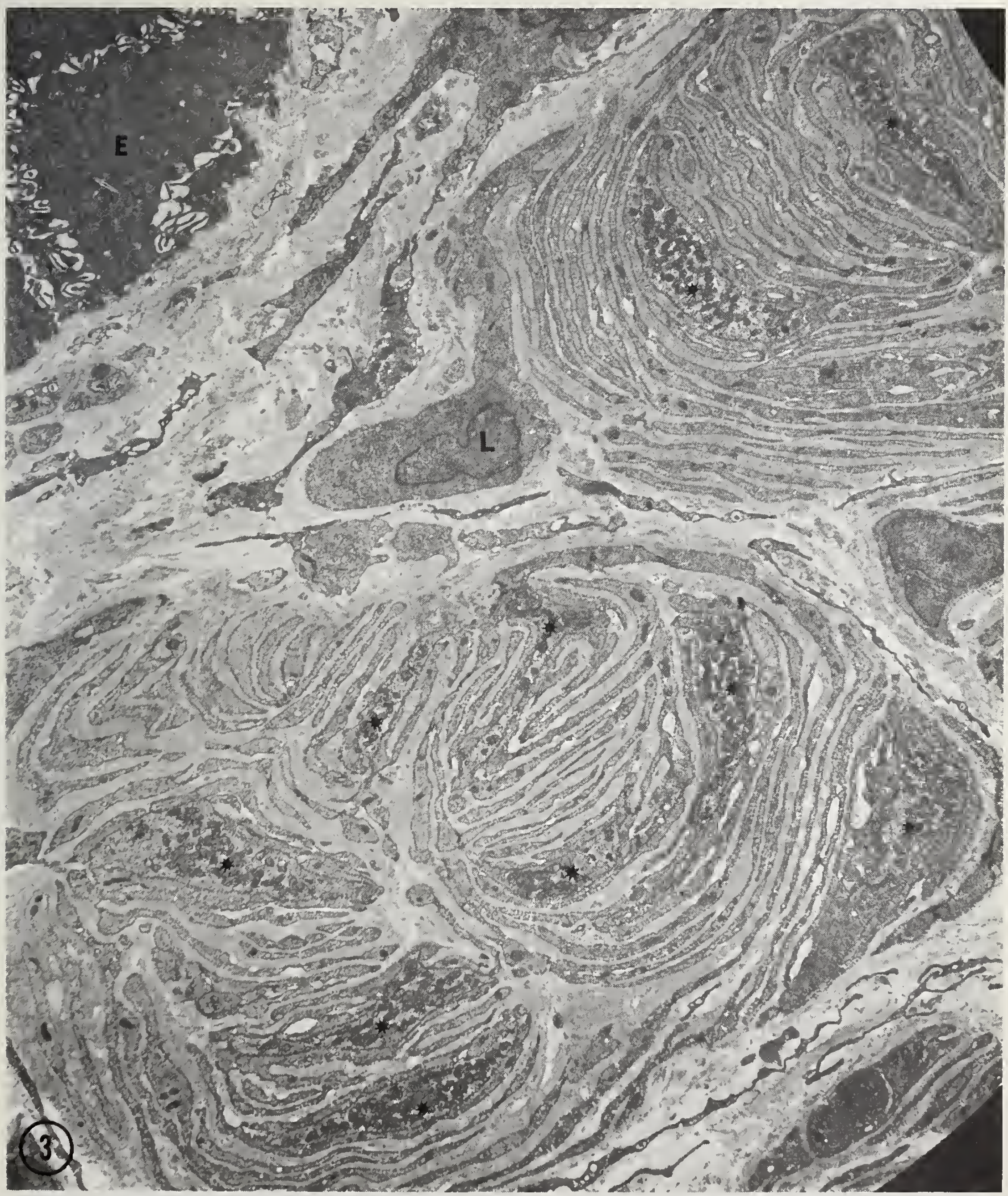


Figure 5–3. This low magnification electron micrograph depicts several parallel loops of the terminal neurite (stars) separated by numerous cytoplasmic lamellae. A nucleus (L) of a lamellar cell is cut in sections and a cytoplasmic process similar to that seen in 5A is present. No distinct capsular cells separate the corpuscle from the overlying oral epithelium (E) or from the connective tissue compartment to the lower left or right. Only scattered fibroblasts are present (see Figure 7). An amorphous filamentous material is present between the lamellae (see Figures 4, 6 and 7). (X4,500).

When the nerve fiber is seen in profile, the lamellae of the lamellar cell provide a cellular investment similar to that provided by any Schwann cell (Figs. 5–5B, 5–6, 5–7). The neurite itself is rarely in direct contact with the connective tissue compartment.

The neurite frequently contains numerous mitochondria as do many sensory nerve endings (Figs. 5–3, 5–5B, 5–6)[21] Unlike some corpuscular receptors no junctional complexes have ever been observed between the plasma membranes of the neurite and its associated lamellar cell. These neurites also contain myelin figures and other lipoidal material (Fig. 5–6).[2,21] Neurofilaments and neurotubules are also present but they lack the precise arrangement found in the more proximal parts of peripheral nerves.

The connective tissue compartment between the lamellae is different from that of typical loose connective tissue. A considerable amount of amorphous filamentous material is present, and scattered collagen fibrils are present (Figs. 5–4, 5–6, 5–7). The collagen fibrils are not regularly arranged as is the case in Pacinian corpuscles.[21] The filamentous material and collagen fibrils merge with the scant basal lamina enveloping each lamella.

A true capsule is not present. Instead flattened fibroblasts form a discontinuous layer at the margin of the corpuscle (Figs. 5–4, 5–7). These cells can occasionally be observed branching, but lack the lamellae and a basal lamina of lamellar cells. Discontinuities in this layer of cells permit direct access from the stromal component of the corpuscle to the stroma of the core of the fungiform papillae.

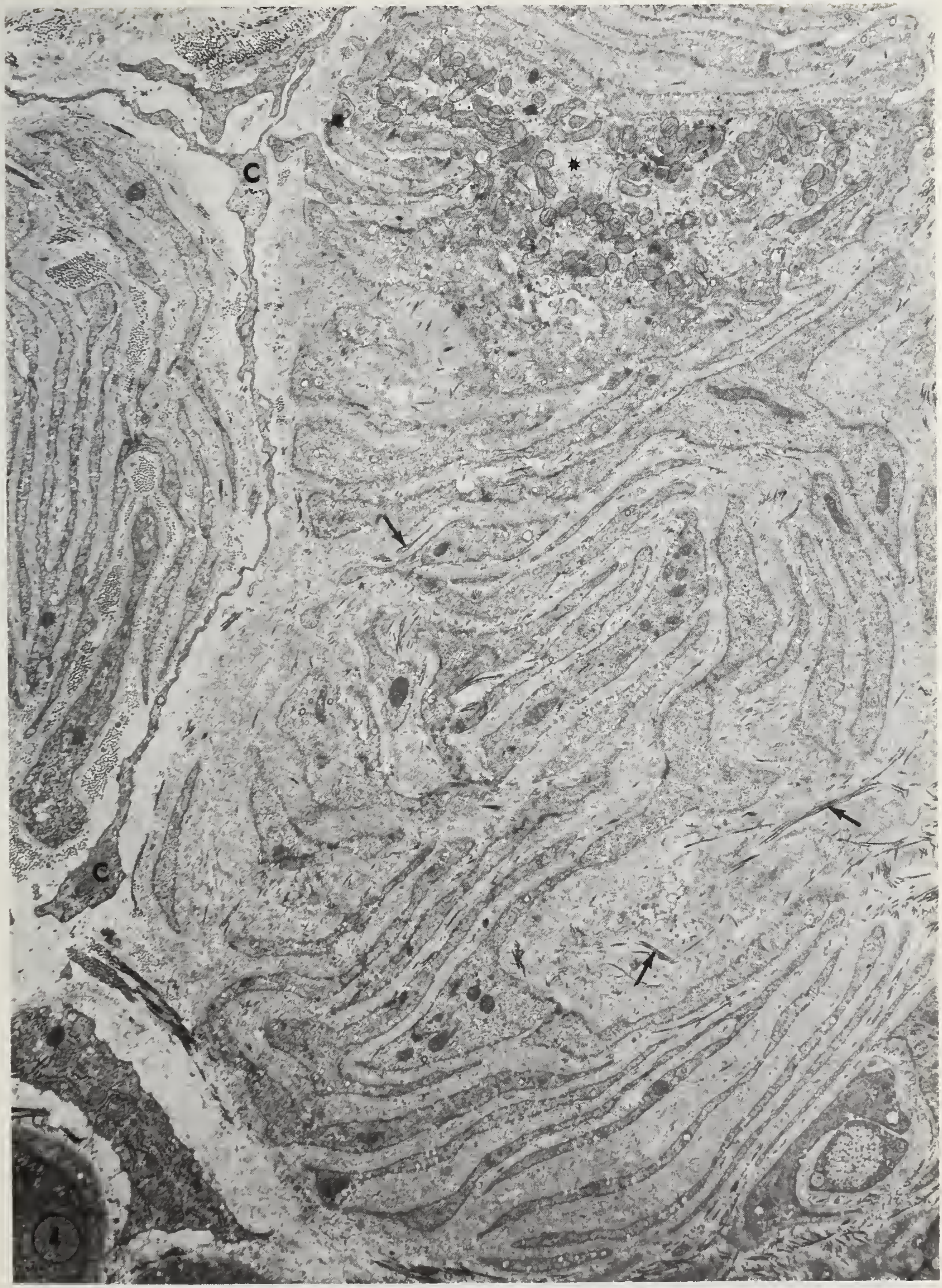
Most Meissner corpuscles are found very close to the overlying oral epithelium. On occasion the endings are present deeper in the connective tissue core of the fungiform papillae. In both locations the ultrastructure is similar.

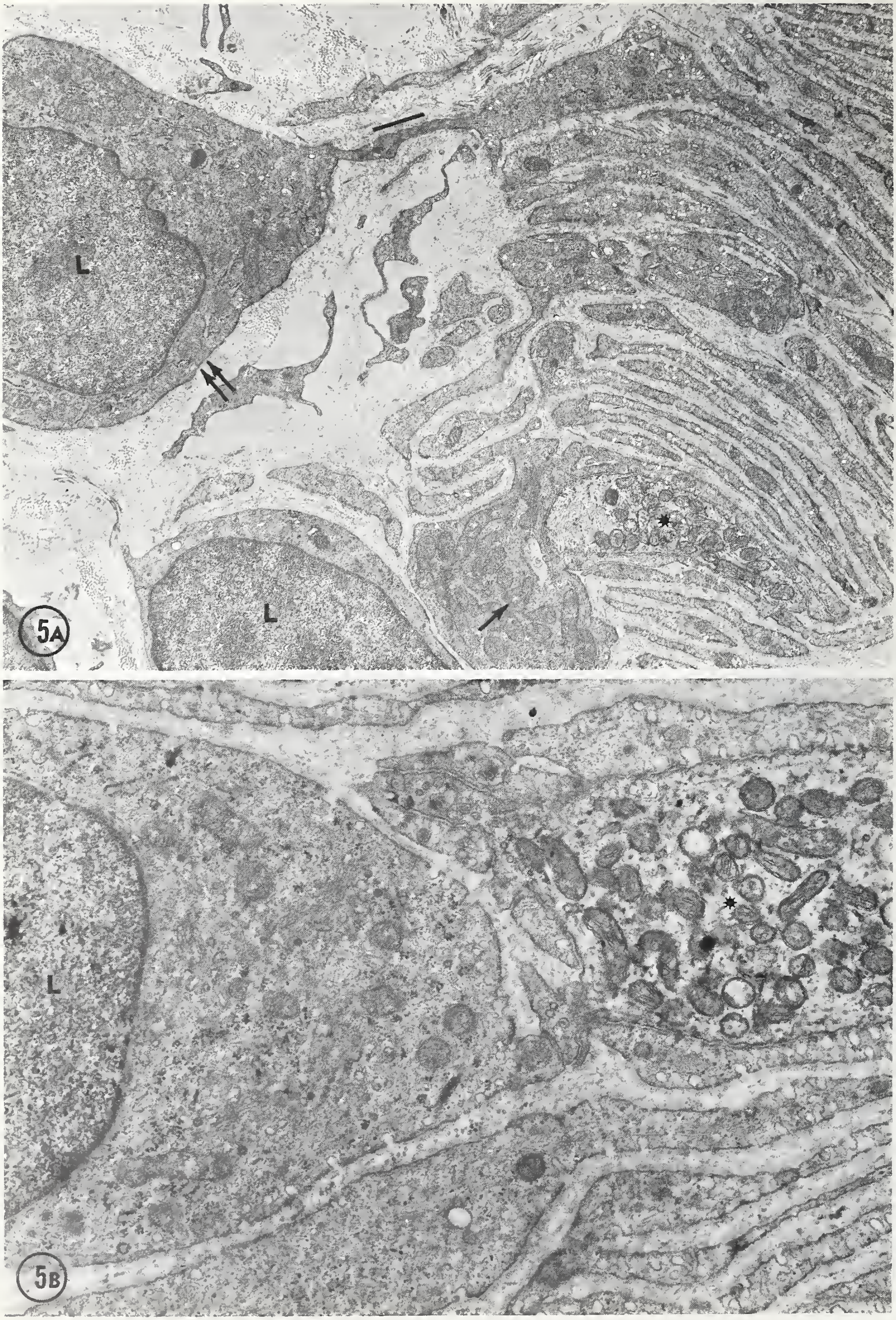
Bundles of unmyelinated nerve fibers are also present in the connective tissue core of the papillae. Some of these may have been autonomic in nature, others could have been gustatory in nature. No effort was made to trace these fibers into taste buds.

Glomerular corpuscles are characterized at a light microscopic level by the characteristic loose coil of the terminal neurite (Fig. 5–2B). The tissue between loops of the neurite does not appear to be successive stacks of lamellae as seen in Meissner corpuscles. Most of these glomerular corpuscles are intimately associated with the overlying oral epithelium; however, some are present deep in the vascular stromal core of the fungiform papillae.

By electron microscopy, the glomerular corpuscles are characterized by a loosely coiled terminal neurite associated with poorly organized processes of lamellar cells (Figs. 5–8A, B). The

Figure 5–4. This electron micrograph is taken from the edge of a Meissner corpuscle. The lamellae surrounding the neurite (*) are more regular than those towards the bottom of the micrograph. The lamellae repeatedly branch, and their basal lamina is difficult to discern from the amorphous, filamentous, interlaminar material. A capsular fibroblast (C) lacks any sign of basal lamina. Bundles of collagen are observed in parallel array to the left and scattered throughout the interlaminar compartment (arrows). (X17,100). ≫


C
*
C
4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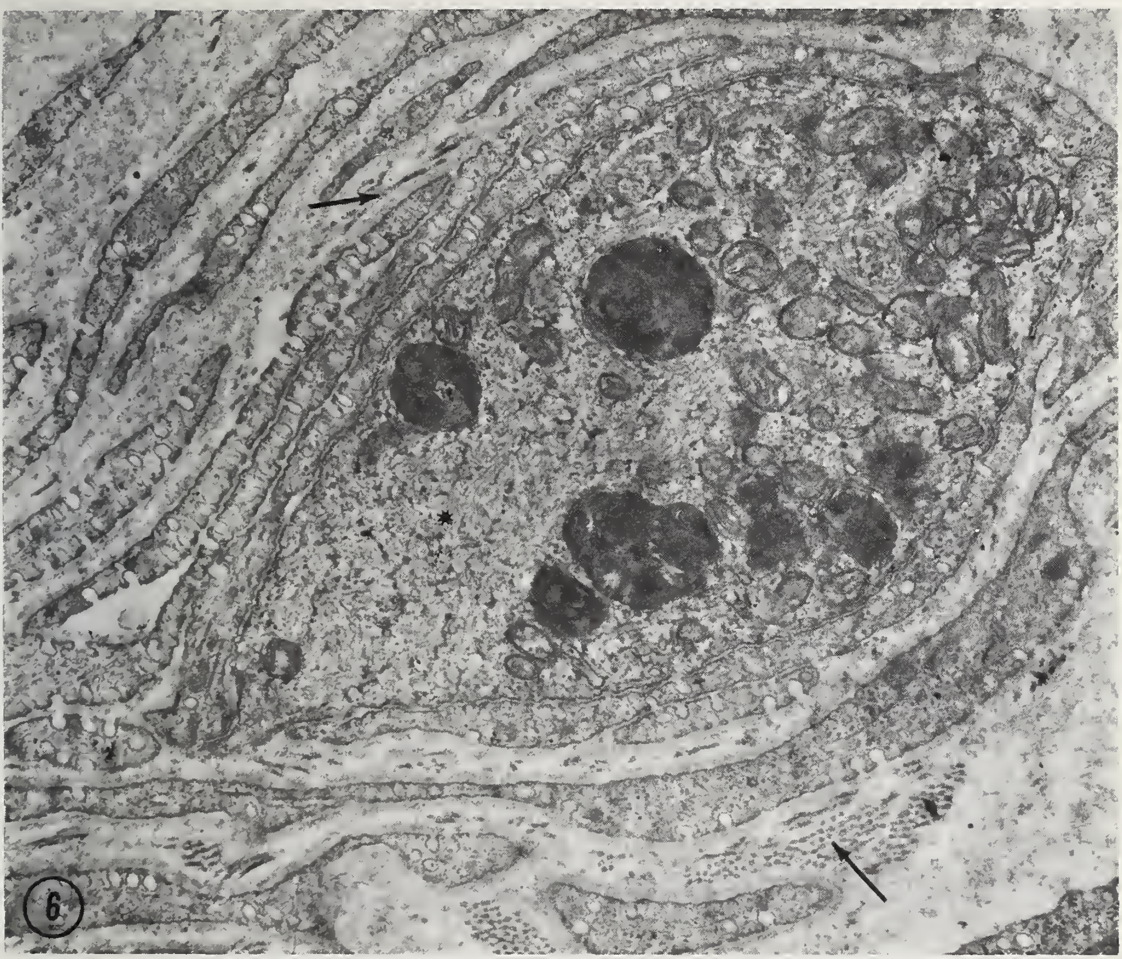

Figure 5–6. The terminal neurites in these sensory corpuscles frequently contain electron opaque masses of presumptive lipoidal material. The neurofilaments and neurotubules (*) are not in precise ordered arrays. The lamellae contain numerous pinocytotic vesicles, including the lamellae applied directly to the neurite. The amorphous component of the interlaminar space can be easily distinguished from collagen fibrils (arrows). (X19,500).

≪ Figures 5–5A and 5–5B. These micrographs depict portions of lamellar cell bodies at two different magnifications. In 5A, the upper lamellar cell (L) extends a slender cytoplasmic projection (bar) into the mass of lamellae surrounding the neurite (*). The lower lamellar cell (L) is not sectioned in direct continuity with the lamellae. Note at the arrow the lamellar cell cytoplasm contain longer mitochondria than those seen in the neurite (*). The basal lamina of both lamellar cells can be discerned even at this low magnification (double arrow). The numerous pinocytotic vesicles characteristic of the lamellae are prominent in this micrograph. (X7,980).

Figure 5B depicts a lamellar cell (L) contiguous with a neurite (*) and its associated lamellae. Both sides of the lamellae have numerous pinocytotic vesicles on their surfaces. A delicate basal lamina invests all lamellae and the lamellar cell. (X19,500).

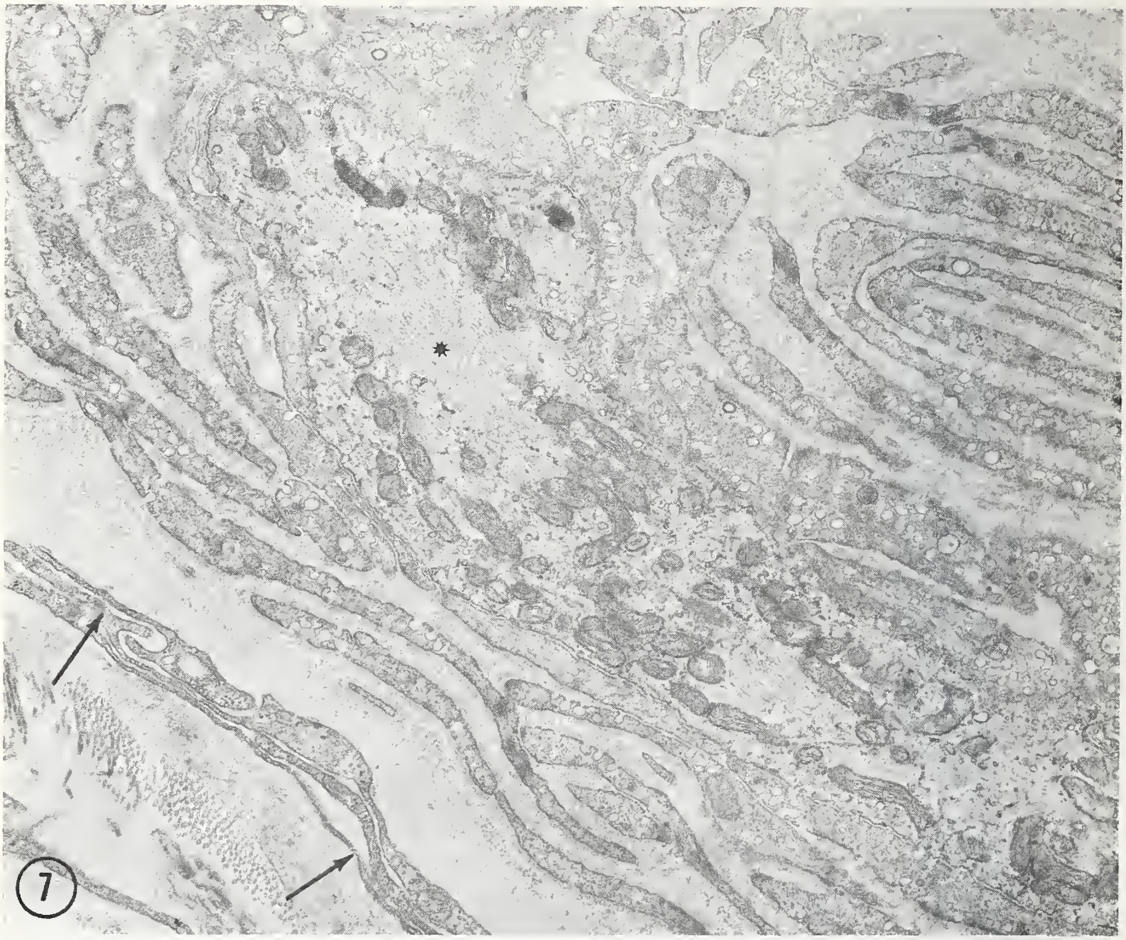

Figure 5–7. This electron micrograph is taken from the margin of a Meissner corpuscle and depicts the difference between the lamellae associated with the terminal neurite (*) and the fibroblasts (arrows) which demarcate the corpuscle almost as a pseudocapsule. The fibroblasts lack any sign of a basal lamina and do not contain pinocytotic vesicles as do the lamellae. (X17,100).

Figures 5–8A and 5–8B. These two micrographs are taken from the same portion of a glomerular corpuscle at low (A) and higher (B) magnification. As compared with the Meissner corpuscles depicted in Figures 3–7, this ending has more profiles of the terminal neurite (*) separated by scant lamellae. It is also closely applied to the overlying oral epithelium (E). In B, the terminal neurite and its lamellar cells form a foot directly contiguous with the overlying epithelium (E). At the arrow, the basal lamina of neurite and lamellar cell is continuous with that of the stratified squamous epithelium. The lamellar cell is similar in all respects to those depicted in Meissner corpuscles (Figures 3–7). (A: X4,500) (B: X19,500).

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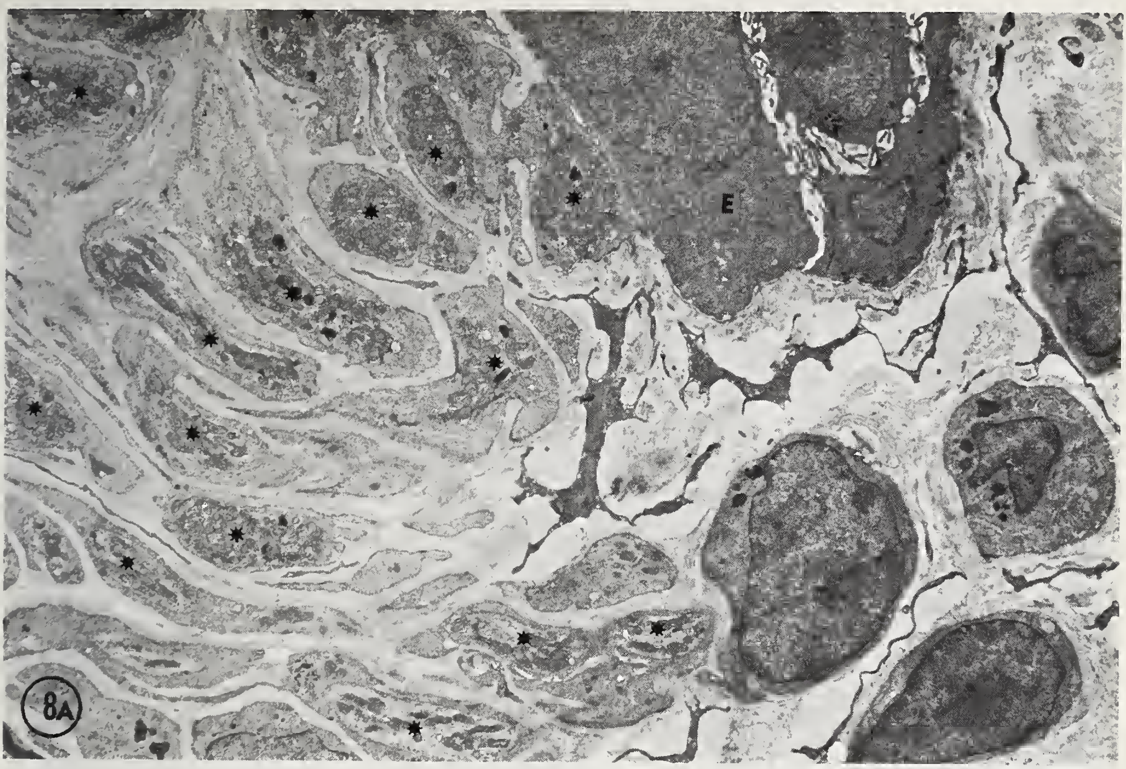
E
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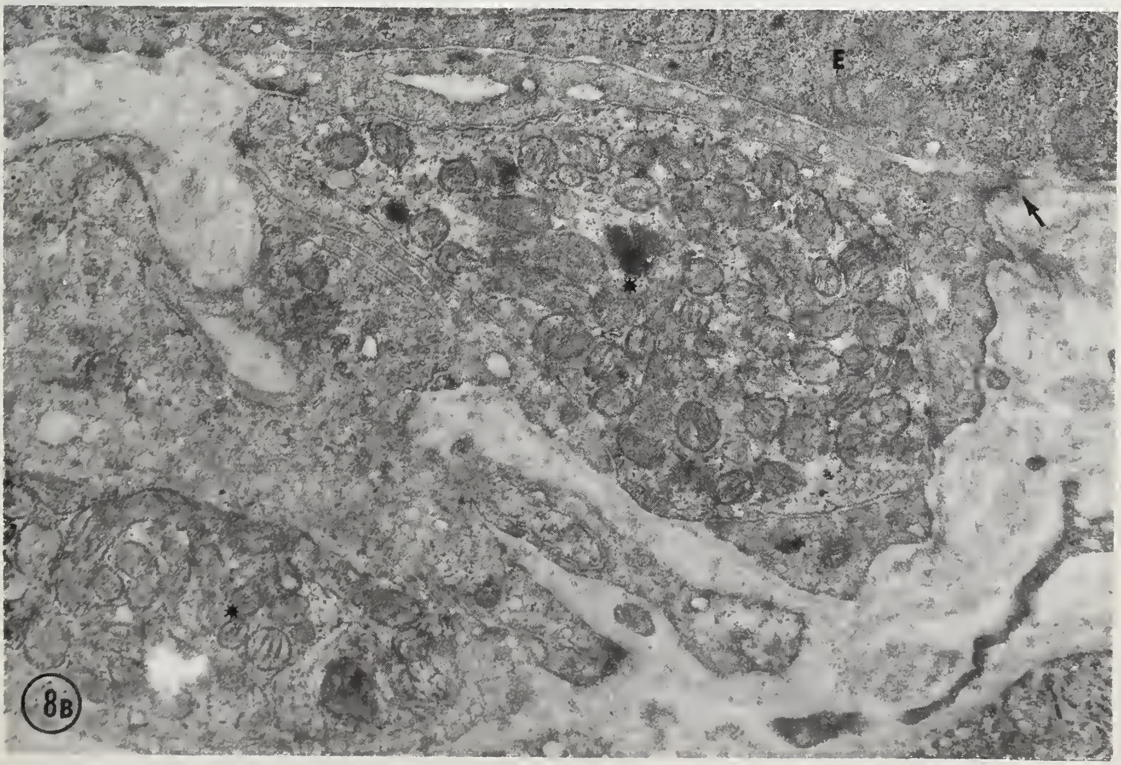
E
8B

lamellae are not seen as stacks of flattened cytoplasmic lamellae as is the case in Meissner corpuscles. The lamellar cells in glomerular corpuscles instead have numerous cytoplasmic extensions some of which envelop the terminal neurite in the manner of a Schwann cell enveloping an unmyelinated nerve fiber. The lamellar cells and their stubby cytoplasmic extensions are enveloped by a distinct basal lamina.

On rare occasion, the glomerular corpuscles have been observed to be in direct continuity with the stratified squamous epithelium (Fig. 5–8B). The terminal neurite and associated lamellar cell extension have been observed abutting directly a basal cell of the stratified squamous epithelium. The foot-like extension of the terminal neurite has a basal lamina that is continuous with that of the oral epithelium. At the point of contact, no basal lamina is present between the neural and oral epithelial component. Although this anatomic configuration resembles that seen when nerves do enter an epithelium,[20] no large sensory neurites and/or Merkel cells have been observed in the lingual epithelium. This absence of Merkel cell neurite complexes is in marked contrast to the situation in other areas of oral mucosa such as lower lip and mucosa of hard palate where numerous Merkel cell neurite complexes have been found (Munger, unpublished observations).

The connective tissue compartment between lamellae and neurite resembles that seen in Meissner corpuscles. Amorphous filamentous material predominates, and only scattered collagen fibrils are present. The corpuscles lack a distinct capsule. Flattened fibroblasts are present at the margin of the corpuscle, but do not form a true continuous epithelial investment as in Pacinian or Herbst corpuscles.[21]

Newborn

The specialized row of fungiform papillae described above in the adult could be recognized in tip of the newborn rhesus monkey tongue. By light microscopy, numerous large (3–8u) nerve fibers could be discerned (Figs. 5–9A, B), but corpuscular endings could not be recognized in these papillae. The presumptive sensory neurites in the core of the fungiform papillae would evidence some looping and coiling, and large polygonal cells were intimately related to the fibers, but they did not resemble mature sensory terminals as seen in the adult.

By electron microscopy, large diameter, presumably sensory neurites were frequently observed (Fig. 5–10). In no instance could they be regarded as corpuscular endings. The neurites were intimately associated with large polygonal cells which resembled Schwann cells in that they had a complete basal lamina investment, and the occasional cytoplasmic processes extended from the cell and enveloped the neurite. Short stubby cytoplasmic processes were also

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Figures 5–9A and 5–9B. These two micrographs are taken from sequential sections of the same fungiform papillae from the tongue of a 3-day-old rhesus monkey. The silver-stained section delineates numerous terminal neurites (arrows) but no discrete corpuscular endings. A taste bud is present at the top of 9A. The neurites are associated with numerous cells, as seen in Figures 10, 11 and 12. The nuclei associated with the terminal neurites are thus presumptive lamellar cells. (Both: X610).

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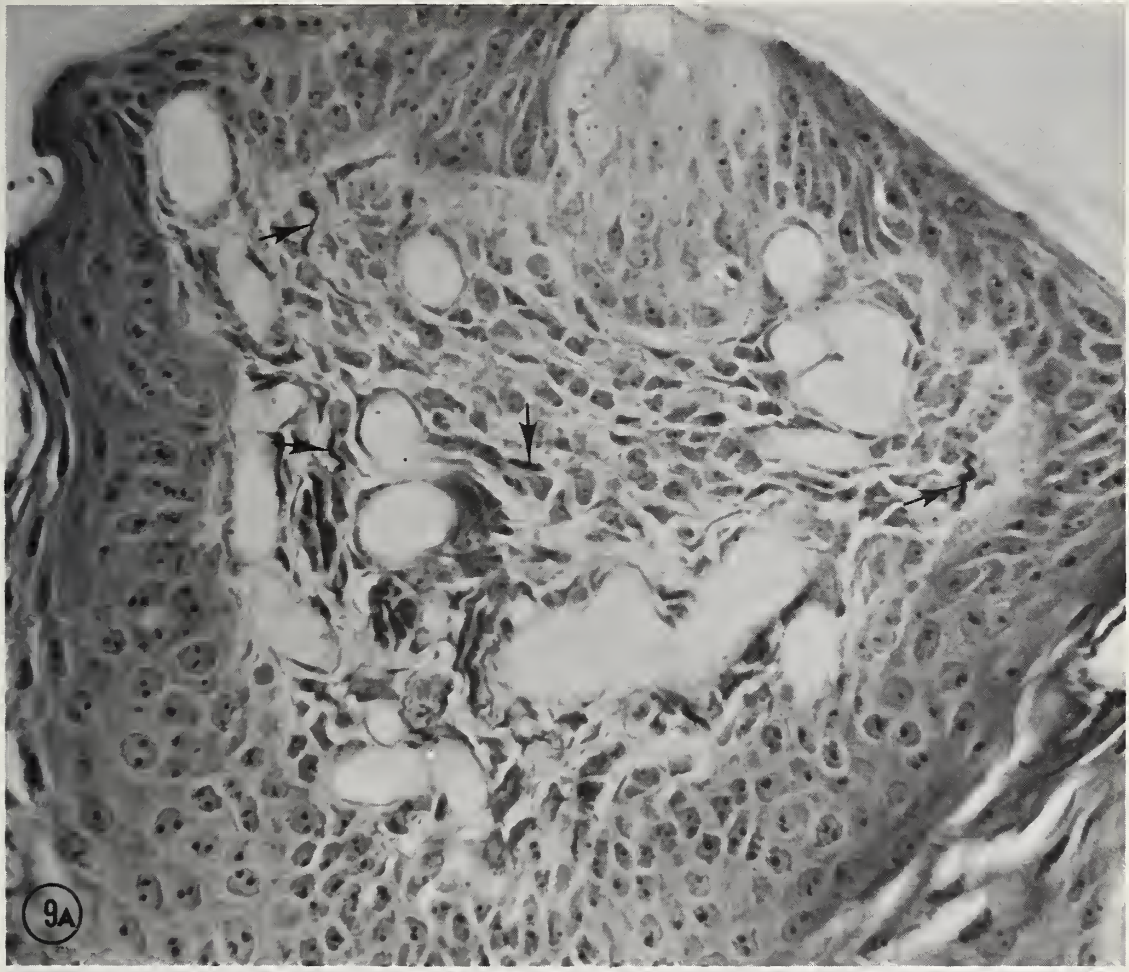
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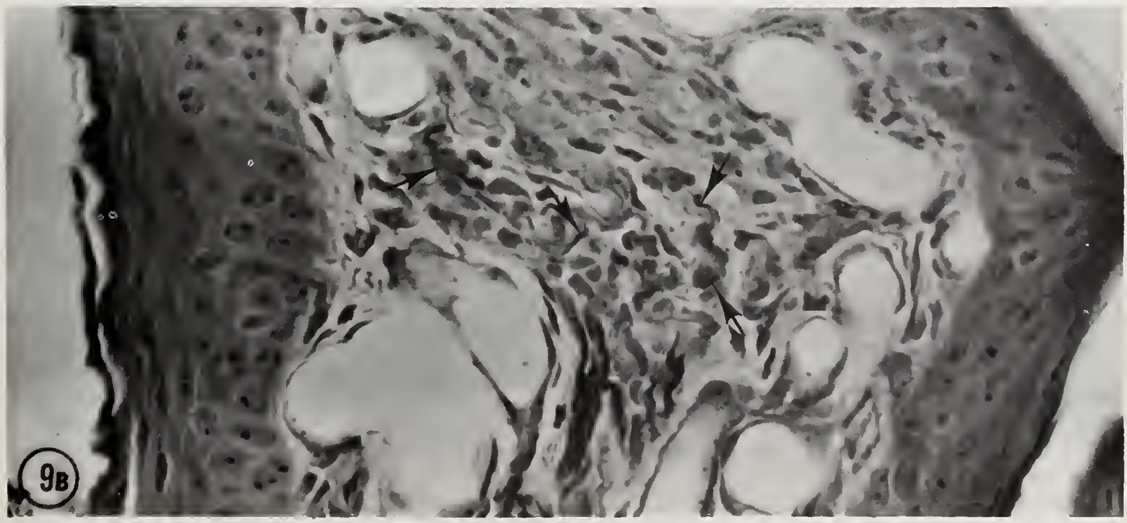
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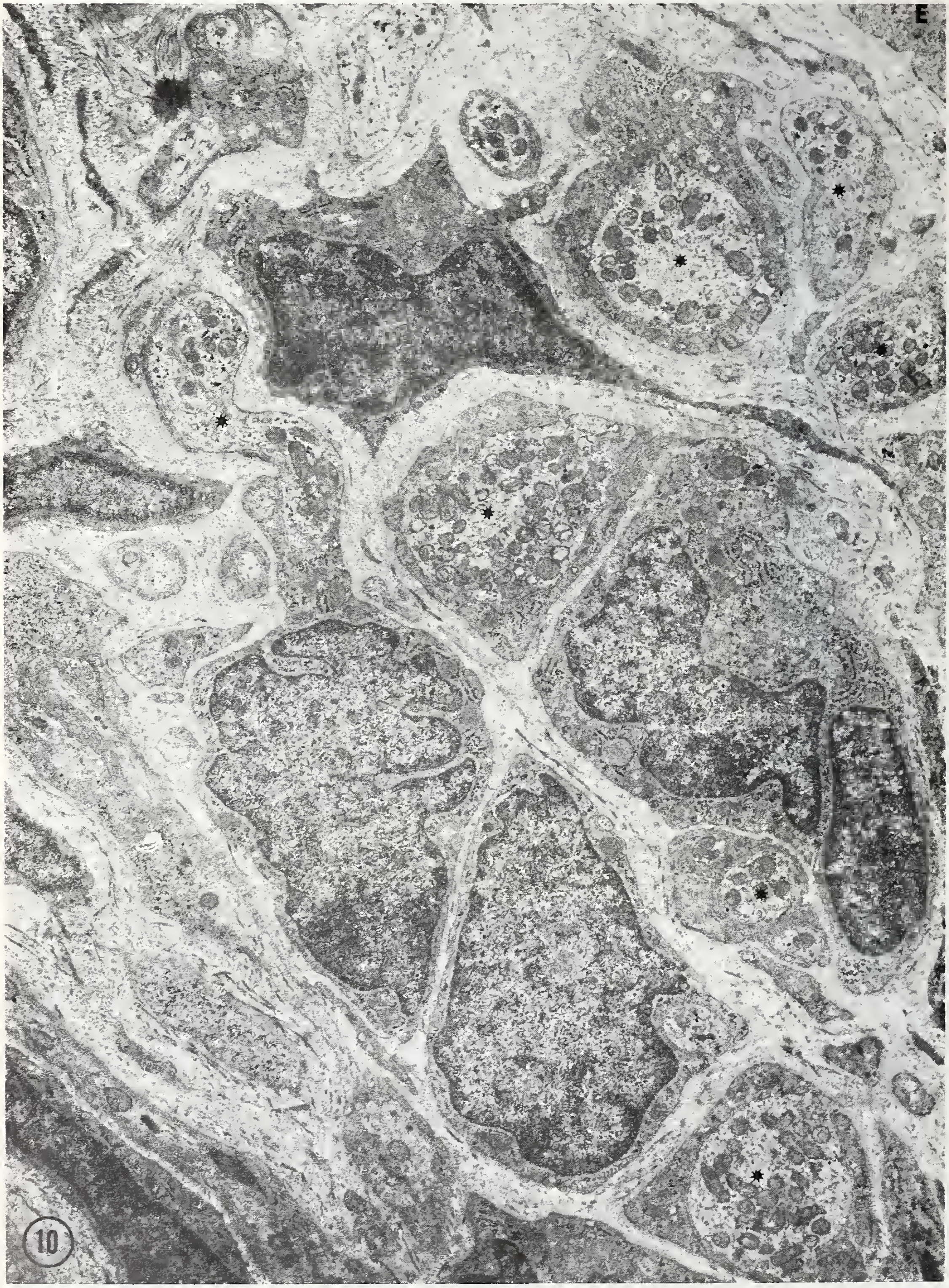
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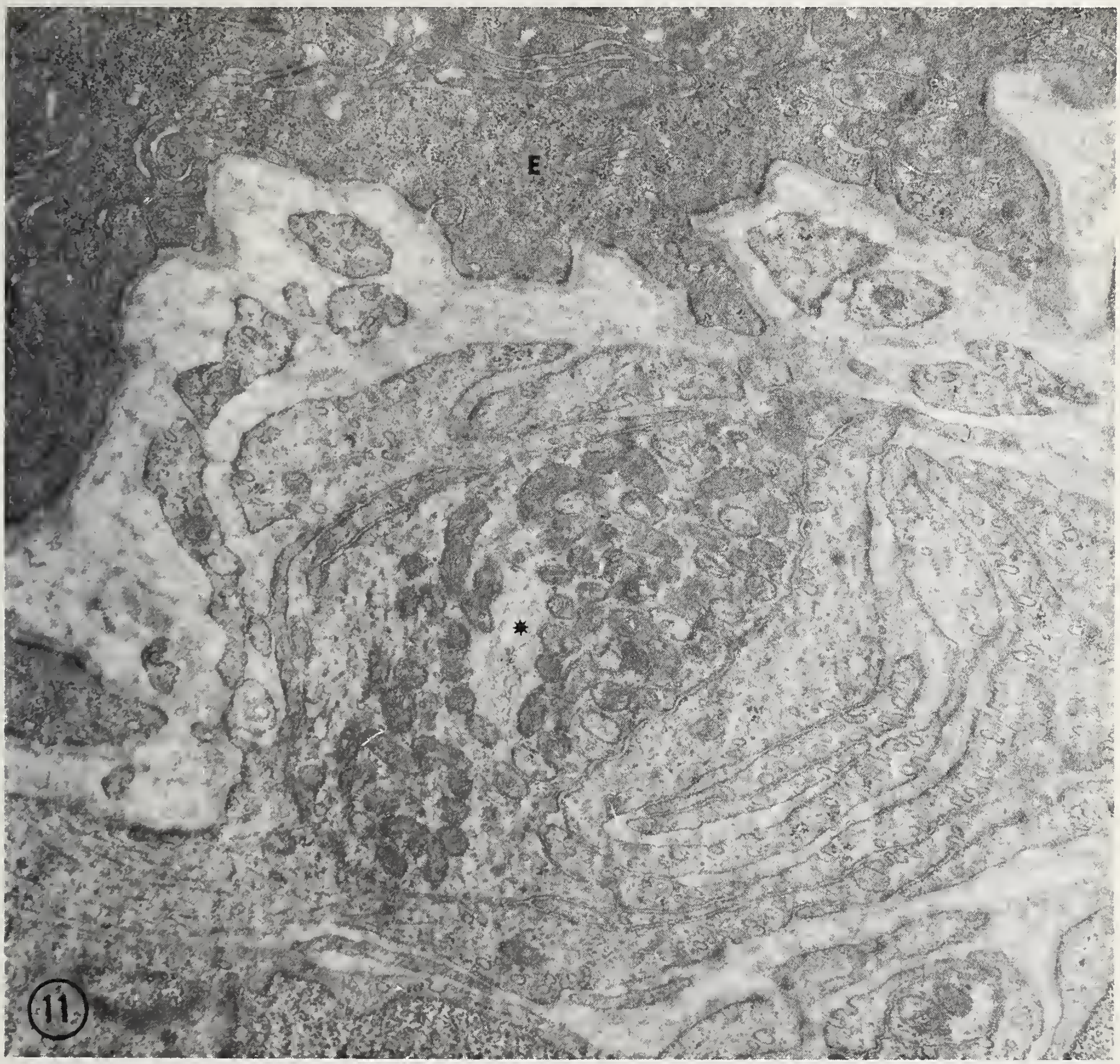

Figure 5–11. A sensory terminal (*) is associated with a few lamellae, but this terminal could not be recognized as a Meissner corpuscle. The lamellae have numerous pinocytotic vesicles, and the terminal is close to the overlying oral epithelium (E). (X19,500).

≪

Figure 5–10. This micrograph is taken from the core of one of the fungiform papillae similar to that in Figure 9. Numerous neurite profiles (*) are associated with large polygonal cells, presumably developing lamellar cells. The overlying oral epithelium (E) can be seen in the upper right. A small bundle of unmyelinated nerve fibers is present in the upper left. (X10,250).

present that were reminiscent of the lamellae in the adult. In rare instances several loosely stacked lamellae were present (Figs. 5–11, 5–12), confirming the impression that these neurites were indeed immature corpuscles, and not merely free nerve endings. While fibroblasts were present in the vicinity of these immature corpuscles, no capsule was present.

Small diameter unmyelinated nerve fibers were also seen, as in the adult, and

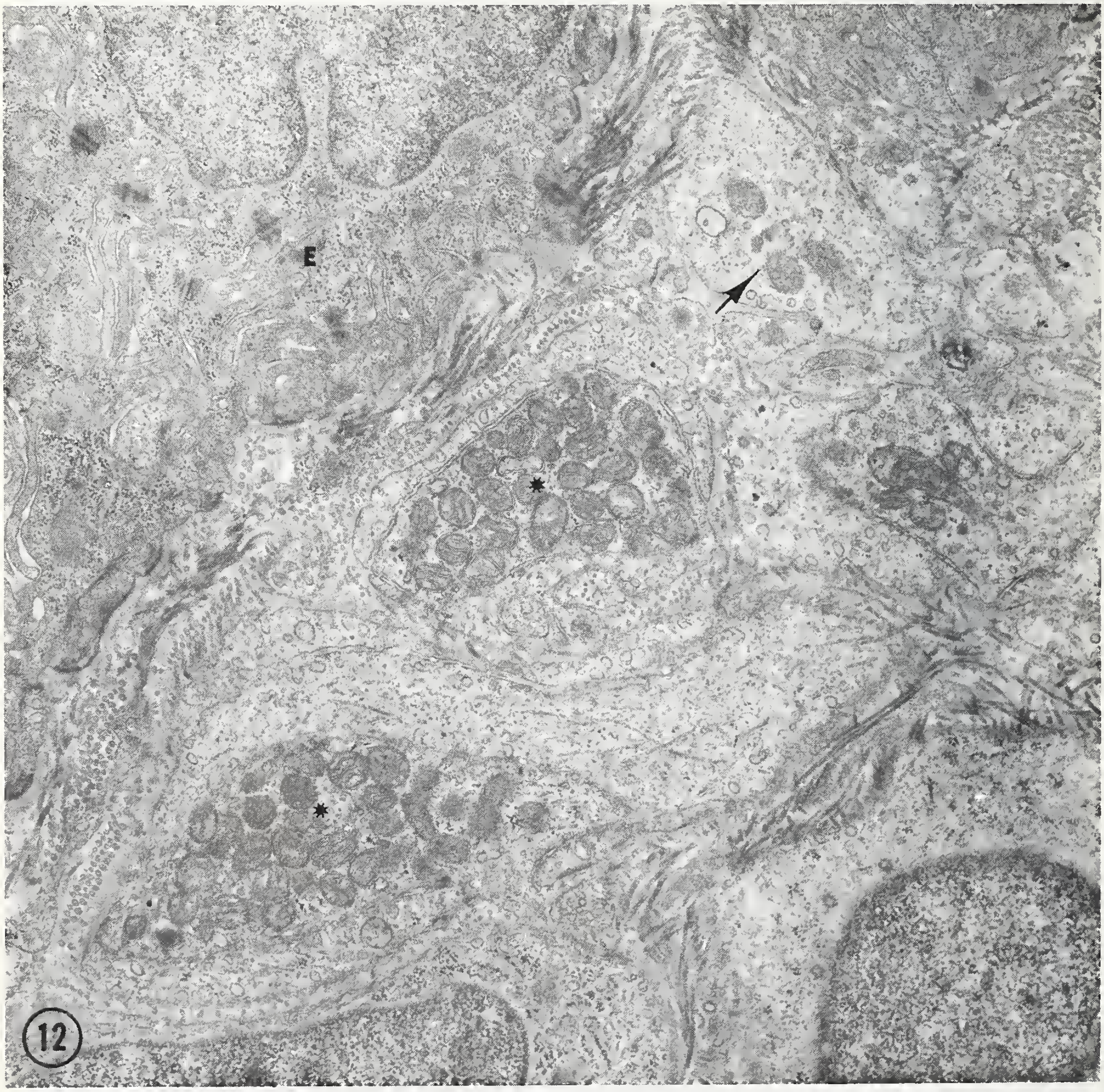

Figure 5–12. Several profiles of large neurites (*) are associated with numerous cytoplasmic processes close to the overlying oral epithelium (E). The cytoplasmic processes have numerous pinocytotic vesicles, and while true lamellae are not present, some processes (arrow) are not associated with neurites in the plane of section. The nuclei of two presumptive lamellar cells are present at the bottom of the micrograph. (X20,200).

were presumed to be autonomic or gustatory in nature.

Juvenile

A limited number of juvenile specimens have been examined to date. By 5 months of age the receptors in the tongue closely resemble those of the adult. In specimens of 3 weeks, 2 months and 3 months of age, the Meissner and glomerular corpusles gradually evidence increasing complexity and closer packing of individual lamellae. The transition is very gradual to the adult state.

SUMMARY

Most of our present knowledge concerning sensory nerve terminals in both a structural and functional sense has been derived from studies on skin, especially hair skin. Our models of slowly and rapidly adapting mechanoreceptors [9,10,34,37] have been derived from hairy skin. While slowly and rapidly adapting mechanoreceptors are present in glabrous digital skin,[11,12,13,16,-23,24,28,37] our knowledge of primate receptors [16,37] is more limited, and we know very little about the oral mucosa. Interpreting the present observations by extrapolating from the bulk of knowledge concerning peripheral cutaneous sensory receptors is fraught with problems and potential errors.

Most corpuscular endings in which lamellar cells and their characteristic cytoplasmic lamellae are present are usually regarded as sensitive rapidly adapting mechanoreceptors.[17] This model is derived from studies on the Pacinian corpuscles and can be successfully extended to include some simple corpuscles.[24] Studies on mechanoreceptors in primate glabrous skin [37] have assumed that Meissner corpuscles are the rapidly adapting transducers in primate glabrous skin. They do resemble a condensation of lanceolate endings of sensory hairs which are rapidly adapting.[22]

The present observations which characterize two types of sensory corpuscles in a group of specific fungiform papillae in rhesus monkey tongue could be important in lingual reflexes in the monkey. The failure to find differentiated sensory terminals in these fungiform papillae in the neonatal rhesus was somewhat unexpected. As presented elsewhere in this symposium,[36] the human newborn tongue is an active organ in terms of tactile reflexes. Preliminary observations by Weiffenbach and Thach (personal communication) indicate that this is not the case in the newborn rhesus. A most interesting question would be what are the functional parameters of the nerve endings in neonatal rhesus tongue. They lack the stacks of cytoplasmic lamellae characteristic of the adult. This should provide us with an opportunity to study the role of the lamellae in an experiment designed by nature by examining the functional parameters of single units of the trigeminal nerve in postnatal rhesus monkeys.

The exact nature of the receptors described as Meissner corpuscles in monkey oral mucosa is somewhat questionable. In almost all respects they resemble Meissner corpuscles of primate digital skin as described by Cauna and Ross. [3,8] The absence of a perineural capsule is not explainable, since similar receptors in the mucosa overlying the hard palate in rhesus monkeys are encapsulated. The ultrastructure of Meissner corpuscles is sufficiently distinctive to permit characterization of those receptors in the tongue (and elsewhere in the oral mucosa) as Meissner corpuscles.

This author has previously pleaded on numerous occasions that we should avoid inventing new nomenclature for old neurologic structures.[20,21,22,25,26] The problem posed by the second corpuscular receptor of coiled nature is not easily resolved. This receptor resembles units that have been called mucocutaneous end organs,[39] glomerular endings,[30,31] Krause endings,[7] and many other terms. They do resemble the genital end bulbs of the rat penis.[25] I would propose that these looped, convoluted receptors associated with loose arrays of lamellae be termed glomerular endings (*glomero*—Latin for "to wind") as proposed by Seto.[30,31] Corpuscular nerve endings would thus be classified into the following structural entities: 1) Pacinian corpuscles; 2) Herbst corpuscles; 3) Meissner corpuscles; 4) simple corpuscles; and 5) glomerular corpuscles. The designation of simple corpuscles follows the suggestion of Poláček [27] that small corpuscles resembling "mini-Pacinian" corpuscles should be termed simple corpuscles. They may evidence some branching but are not coiled or looped. Seto [30,31] suggested the term glomerular endings for complex looped and twisting nerve terminals seen in oral mucosa. Genital end bulbs [25] are thus an example of a glomerular corpuscle. The distinction that I have made in the past [21] on the presence or absence of a capsule is certainly not relevant based on the present observations. The corpuscular endings in primate oral mucosa appear to be demarcated by a capsule by light microscopy, although a capsule could not be defined by electron microscopy. Connective tissue cells which would appear to be capsular in nature are also seen in glomerular corpuscles in the rat penis.[21]

All corpuscular endings described to date by electron microscopy have as a common denominator the presence of lamellar cells.[21] A given type of corpuscular ending is only based on the geometry of the terminal neurite and its associated lamellar cells.

Any understanding of the functional parameters of these various corpuscles must await future physiologic studies. As noted above, the Pacinian corpuscle has been a model for both anatomic and physiologic studies.

Recently the Herbst corpuscle [5,6] as well as the simple corpuscles of raccoon digital skin [24] have been shown to be physiologically similar to Pacinian corpuscles. The possibility exists that *all* corpuscular nerve terminals might be rapidly adapting mechanoreceptors. The oral mucosa appears to be an ideal area in which to test this hypothesis.

Acknowledgements: The author wishes to acknowledge the valuable assistance in the preparation of light and electron micrographs of Katharine W. Beard and Daniel C. Biemesderfer.

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DISCUSSION

KENSHALO: So much of the discussion had centered around mechanoreceptors and myelinated fibers that I was going to ask Dr. Munger about the temperature sensitivity of the tongue. But, he has just explained the bias, the reason for it, and acknowledged that the tongue has thermally sensitive units.

MUNGER: I have discussed only an area where we have lots of nerve endings of a given type. I see other endings in the mouth that are like this and there are certainly many, many other nerves but we have indeed biased our sample towards these corpuscular endings.

THACH: Simple intraepithelial nerve endings, which are apparently not associated with taste buds, have been observed in the lingual epithelium of the human fetus.[1] Did you observe such endings in the fetal monkey?

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[1] Seto, H.: Studies on the Sensory Innervation, Edit. 2, Springfield Thomas, 1963.

MUNGER: We have not observed intraepithelial nerve endings in monkey tongue to date other than gustatory fibers.

THACH: In terms of function, how might intraepithelial endings be expected to differ from complex subepithelial endings, such as you have described?

MUNGER: They might be slowly adapting as receptors compared to rapidly adapting corpuscles. Most complex corpuscles described to date are characterized physiologically as rapidly adapting.

STEINER: Do you have physiological evidence for your suspicion that the described lamellar structure of the nerve-endings are related to the process of transduction? I have the impression that your lamellar structures are analogous in micro-dimensions to those in the Pacinian corpuscles. So far, we don't know the physiological role of the lamellar structures in the Pacinian corpuscle. Hence we cannot exclude the possibility that these lamellae are involved in the transduction process, possibly by some kind of membrane discharges due to displacement.

MUNGER: The role of the lamellar cells in a Pacinian corpuscle is thought to be related to depolarization or hyperpolarization of the terminal in response to compression,[1] or perhaps to its rapidly adapting characteristics.[2] The role of such cells in these corpusclar receptors is not known.

GOBEL: Both of the types of receptors you described are associated with myelinated axons. It would appear that the role of the anatomically defined unmyelinated axons keeps diminishing as you work out more and more of these receptors. Do you envision a role for unmyelinated axons?

MUNGER: Small diameter myelinated or unmyelinated fibers one usually thought to be terminal and/or pain fibers. We simply do not know, for sure, the function of an unmyelinated fiber. They can also be autonomic in nature.

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[1] Ozeki, M., and Sato, I., *J. Physiol.*, 180:186–208, 1965.

[2] Lowenstein, W.R., and Mendelson, M., J. Physiol., 177:377–397, 1965.

Chapter 6

MORPHOLOGIC ZONES OF THE HUMAN FETAL LIP MARGIN

BRADLEY T. THACH

INTRODUCTION

The lips are prehensile structures adept at grasping objects by apposition of lip to lip or of lip to tongue. Like other organs for manipulation, they are exquisitely sensitive to stimuli. The lips are more sensitive than the fingertip or even the tongue to light touch.[14] They rank close to tongue tip in temperature sensitivity and in two point discrimination.[15] The lips develop sensory and motor functions early in fetal life. Hooker and Humphrey have demonstrated reflex facial movements to stimulation of the lip in fetuses as early as 7.5 weeks menstrual age.[16] This occurs before responses can be elicited by stimulation of the extremities.

In view of these highly developed functional attributes, the anatomy of the lips and particularly their embryology is of considerable interest. As compared to the finger or tongue, however, the embryology of the lips has received little attention in recent years. This was not always so. In 1863, Lushka's[19] discovery of small "villi" on the lips of newborn infants ("pars villosa") stimulated a number of studies describing the gross anatomy, and histology of these interesting structures.[21,24,27] The "villi" were said to be attached to elevated ridges of epithelium on the upper and lower lips. These ridges were presumed to be functional adaptations and were termed "sucking pads".[35] Anson[1,2] described the comparative anatomy of the lips in detail. In certain fish, numerous small labial papillary structures carry taste buds and are therefore known to have sensory function. Anson thought the primary function of lip papillae in other animals (bats) was mechanical. He concluded that labial papillary structures in all species are homologous with the teeth of selachian fishes. Later Wherry and Anson[36] observed that the "villi" of the human "pars villosa" were in large part artifacts resulting from fetal death and maceration in amniotic fluid. Under such conditions the connective tissue papillae of the lamina propria appear to project above the lip surface as a result of the loss of surrounding epthelial cells by desquamation. This observation did

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Acknowledgement: The author is grateful to Mary Alice Larsen who prepared most of the histologic materials, also to a number of persons who have reviewed my material and have contributed to the preparation of this manuscript especially Bryce Munger, James Bosma, Richard Gruelich and Howell Archard. I am especially indebted to Dr. Ronan O'Rahilly and the Carnegie Institute of Washington for use of their embryology collection and for donating tissue from 4 specimens in the collection. Without the cooperation of the Departments of Pathology of the District of Columbia General Hospital, Bethesda Suburban Hospital and the U. S. Naval Medical Center, Bethesda, Md., this study would not have been possible.

much to detract from earlier studies, although mention may yet be found of lip "villi" and of the "pars villosa" in relatively recent[11,26,28] or even current anatomy texts.[29] It is odd that Anson, himself, retained the term "pars villosa" after this discovery. The notion that the labial "sucking pads" are specific adaptations for suckle feeding[5,22,30] which atrophy from disuse after infancy[24,27] also still persists, although there has been little objective evidence to support this view.

This chapter reviews the embryology of the lip integument. My interest has been focused on the early differentiation of the epithelium into several morphologically distinct zones. The first part of the chapter describes the histology* and is followed by observations of living infants.

EARLY DEVELOPMENT

Between 6 to 8 weeks gestational age*, the epithelial cells on the appositional margin and inner surface of the lips divide and form a layer 10 to 12 cells

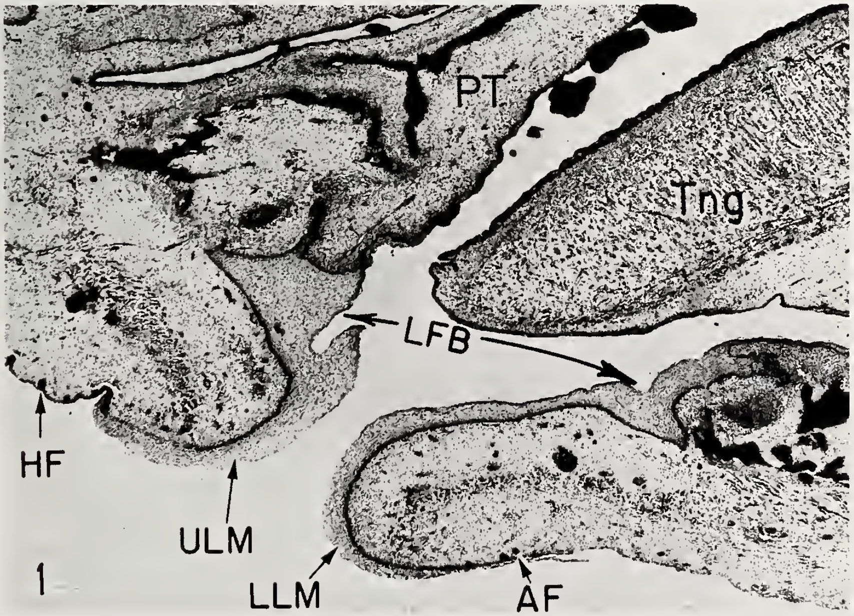

Figure 6–1. Sagittal section of the oral region of a 54 mm CR fetus (11.5 weeks). The thick layer of epithelial cells found in the lip furrow bands (LFB) extends onto the upper and lower lip margins (ULM and LLM). The tongue (Tng) and palate (PT) are covered by relatively thin epithelium. Hair follicle primorida (HF) mark the transition to skin (periderm). H and E, x 20.

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*Gestational (menstrual) age for small fetuses was estimated from the crown rump length using the data of Streeter.[31] In larger fetuses (CR>220 mm), age was estimated by averaging estimates from 50th percentile means for head circumference, weight and crown heel length using the data of Usher et. al.[33]

*These observations and illustrations are from a study of 19 unmacerated fetuses from 12 weeks menstrual age to term. Some of these were stillborn and some survived for several days after birth. Supplemental information on early development was gained from the study of mounted serial sections of 13 fetuses, (21–78 mm CR) in the embryological collection of the Carnegie Institute of Washington.

deep. This epithelial layer is connected with a similar thicker layer of cells in the labial sulcus (lip furrow band). Elsewhere, the skin and the mucosa (outer lip, palate, tongue) at this stage of development is still covered by a double layer of epithelial cells. At 11 weeks hair follicles develop on the surface of the lip from ingrowths of epidermis beginning at the point where the thickened epithelium ends and the double layer of cells (periderm) begins (Fig. 6–1). At 17 to 19 weeks, small thin-walled blood vessels grow upward from the submucosa to come into contact with the epithelium along the appositional margin of the lips. They continue to grow upward and appear to lift the basement membrane to form small papillae (Fig. 6–3). By 25 weeks these papillae, with contained vascular loops, project far up into the surrounding epithelium (Fig. 6–4). The appearance of these vascular papillae divides the margin of the lip into three areas or zones: an outer glabrous zone, a middle papillary zone and an inner (vestibular) zone which at this time has no papillae (Fig 6–2). The epithelium overlying the papilla increases in thickness while that of the emerging glabrous zone fails to do so. A shallow external groove forms where the glabrous and papillary zones meet. At this stage the superficial cells of the glabrous zone are already becoming flattened, showing a tendency towards keratin formation.

By the 17th week of gestation, nerve fibers can be seen projecting toward the epithelium of the lips. Many terminate in networks about hair follicles (Fig 6–5). In the glabrous zone, beaded nerves which are small in diameter remain below the epithelium in close association with blood vessels (Fig. 6–6). Small in diameter nerve fibers are seen (23rd week) in the mucosal papillae of the papillary zone which terminate close to the epithelial basement membrane (Fig. 6–7 and 6–8).

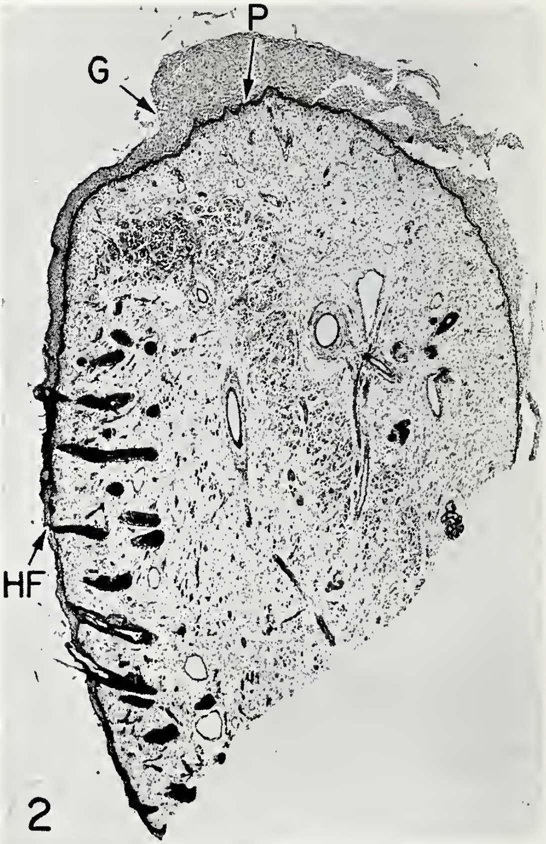

Figure 6–2. Sagittal section of the lower lip of a 139 mm CR (18 week) fetus. Formative connective tissue papillae (P) of the lamina propria appear as small indentions in the basement membrane. A shallow groove (G) in the epithelium marks the boundary between papillary and non-papillary areas. Hair follicles (HF) appear on the outer lip. H and E, x 24.

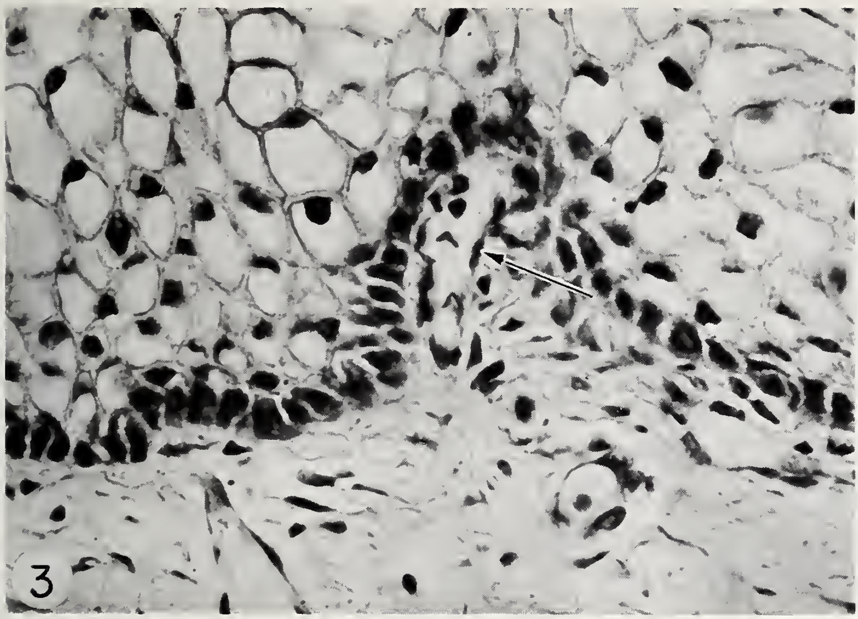

Figure 6–3. Formative papillae in the margin of the lower lip from a 145 mm CR (18.5 week) fetus. A vessel (arrow) fills the center of this early papilla. H and E, x 560.

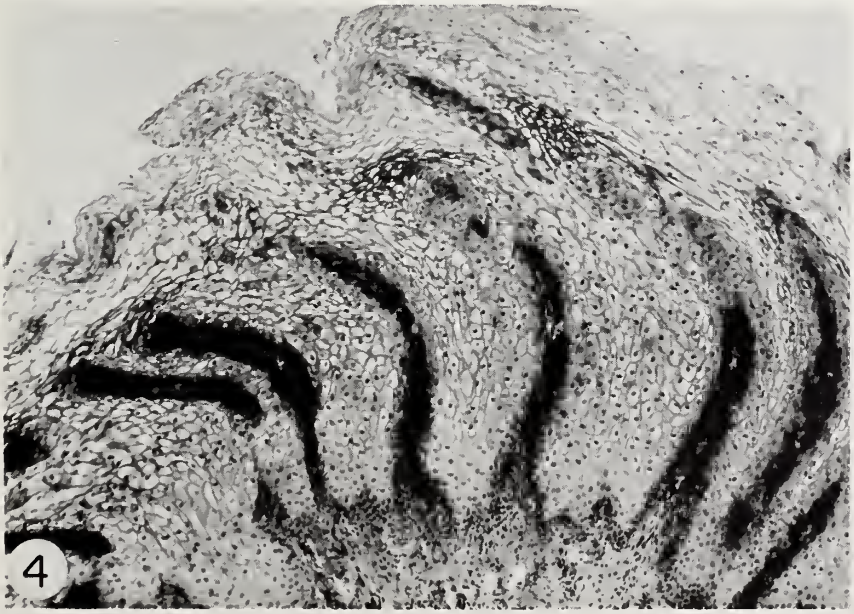

Figure 6–4. Epithelium with well developed papillae from the lower lip (papillary zone) of a 29.5 week live-born fetus. H and E, x 90.

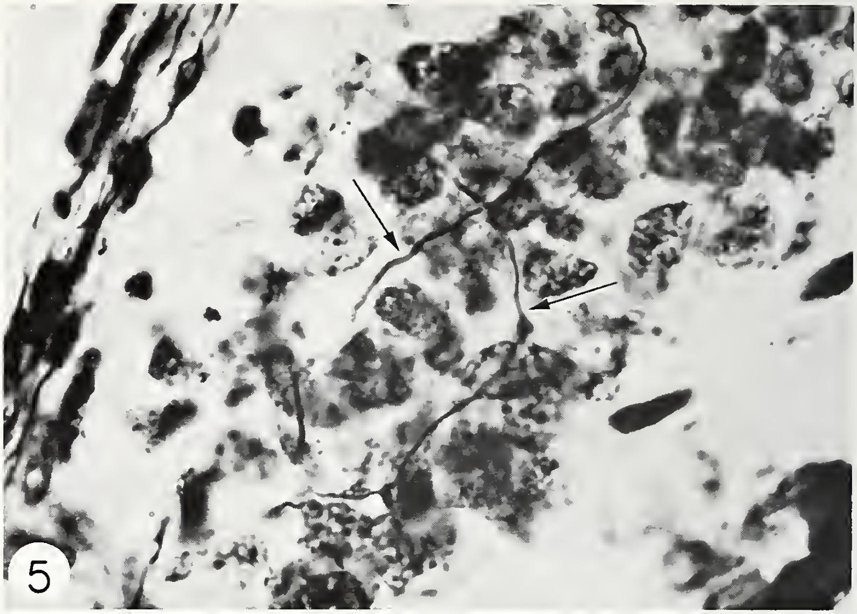

Figure 6–5. Nerve fibers (arrows) about cells of a hair follicle in the hairy cutaneous zone of the lower lip of a 205 mm CR (23.5 weeks) fetus. Bodian, x 1400.

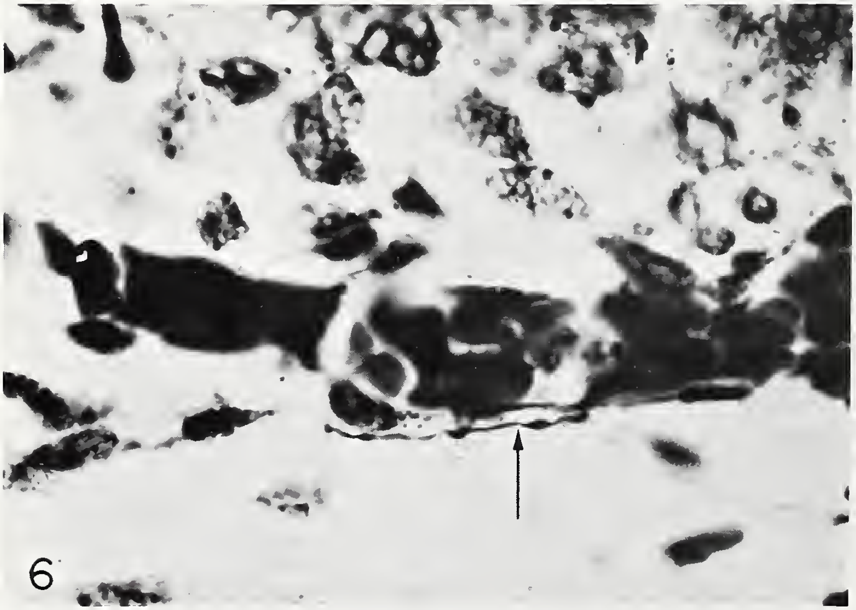

Figure 6–6. Nerve fiber (arrow) following vessel in the glabrous zone of the lower lip of a 205 mm CR (23.5 weeks) fetus. Bodian, x 1400.

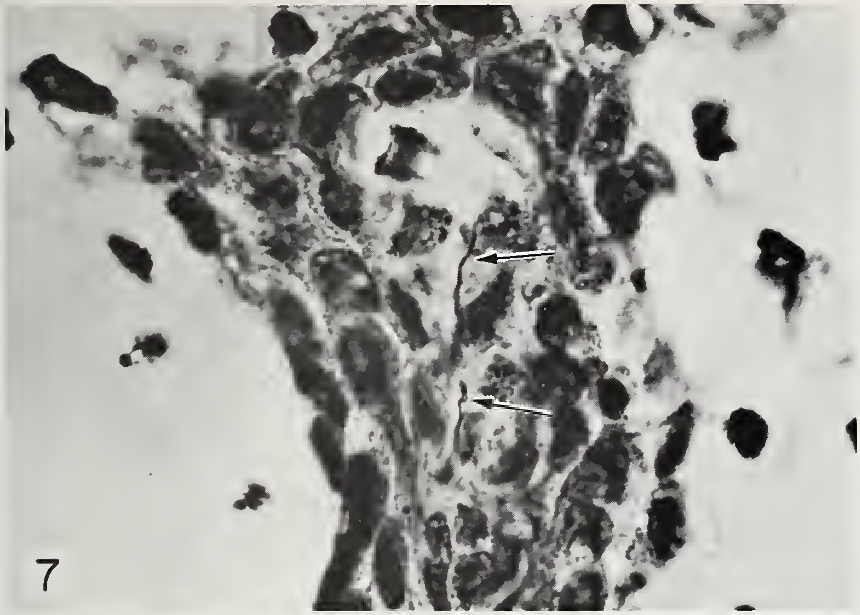

Figure 6–7. Nerve fiber (arrows) which appears to terminate within a formative papilla in the papillary zone of the lower lip of a 210 CR (24 weeks) fetus. Bodian, x 1400.

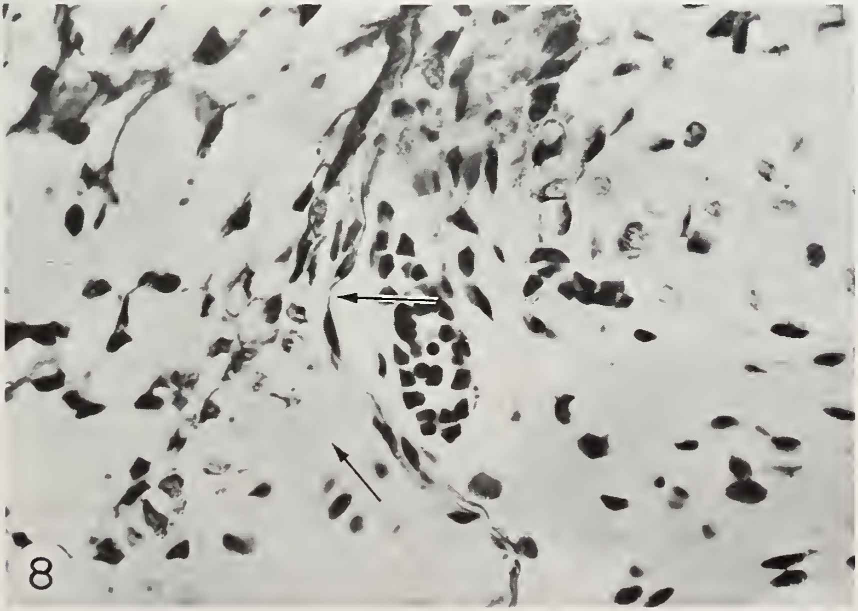

Figure 6–8. Branching nerve fiber which extends into the base of a papilla in the papillary zone of the upper lip of a 27.5 weeks fetus. Bodian, x 560.

LATE DEVELOPMENT

The characteristic features of the epithelium covering the lip are well developed by the 30th week of gestational age (Fig. 6–9). The hairy cutaneous zone is covered by well keratinized epithelium 50–100 microns thick. The epithelial cells are not vacuolated and do not stain well with the periodic acid schiff (PAS) treatment indicating relative lack of glycogen. The junction between epithelium and dermis is uneven but there are no well developed dermal papillae. Small vessels, usually less than 10 microns in diameter, rarely appear close to the epidermal basement membrane (Fig. 6–10a). Superficial nerves are mainly associated with hair sheaths and few are seen to approach the epithelium between follicles.

The glabrous zone has no hair follicles. Sebaceous glands may persist in the lamina propria, especially in the upper lip. The epithelium is thicker (100–150 microns) and on the surface, nuclei of flattened epithelial cells are retained (parakeratin formation). In term fetuses this zone may be fully keratinized. The epithelium is not vacuolated and does not stain for glycogen. Small irregular papillae (up to 100 microns in length) may be present and often contain a small vessel (5–10 microns in diameter) (Fig. 6–10a). Small nerve fibers rarely terminate close to the epithelial basement membrane.

There is usually a shallow groove in the superficial epithelium at the inner margin of the glabrous zone which separates this zone from the adjacent papillary zone (Fig. 6–11). Here the parakeratinized epithelium is replaced by nonkeratinized epithelium (Fig 6–10). Epithelial cells between papillae become larger and appear to be distended by a large perinuclear vacuole which is PAS positive in most specimens. This PAS positive material is thought to be glycogen since it is removed by pre-treatment with diastase and does not stain with mucicarmine, colloidal iron or alcian blue (Fig. 6–11). The basal layer of cells loses its vacuoles and glycogen as the fetus approaches term. However, vacuolated cells persist near the surface between papillae. In stillborn macerated specimens, these superficial cells tend to be sloughed out of the spaces between epithelial papillae, so that the papillae appear to protrude above the epithelial surface. This also seems to occur to a slight extent during fixation and processing of fresh tissue since detached epithelial cells are frequently seen close to the surface in mounted sections. In the Negro fetus, pigment laden cells in the basal stratum are numerous, expecially in the tips of papillae.

As in the younger fetus, the papillae of term born infants are well developed. They are 350 to 900 microns in length x 60 microns in width. The papillae on upper and lower lips are similar. At the angles of the mouth these long papillae are restricted to a narrow band which passes onto the buccal mucosa and extends back to the palatoglossal fold. Each papilla contains a single thin-walled vessel which forms a hairpin loop close to the papillary tip (Fig. 6–12a and b). This vessel is usually filled with erythrocytes making injection of contrast material unnecessary for adequate visualization (Fig. 6–12a and b).

The ascending and descending limbs of the vessel fill the papilla, leaving little room for other structures. Each limb is of approximately equal diameter. Each is

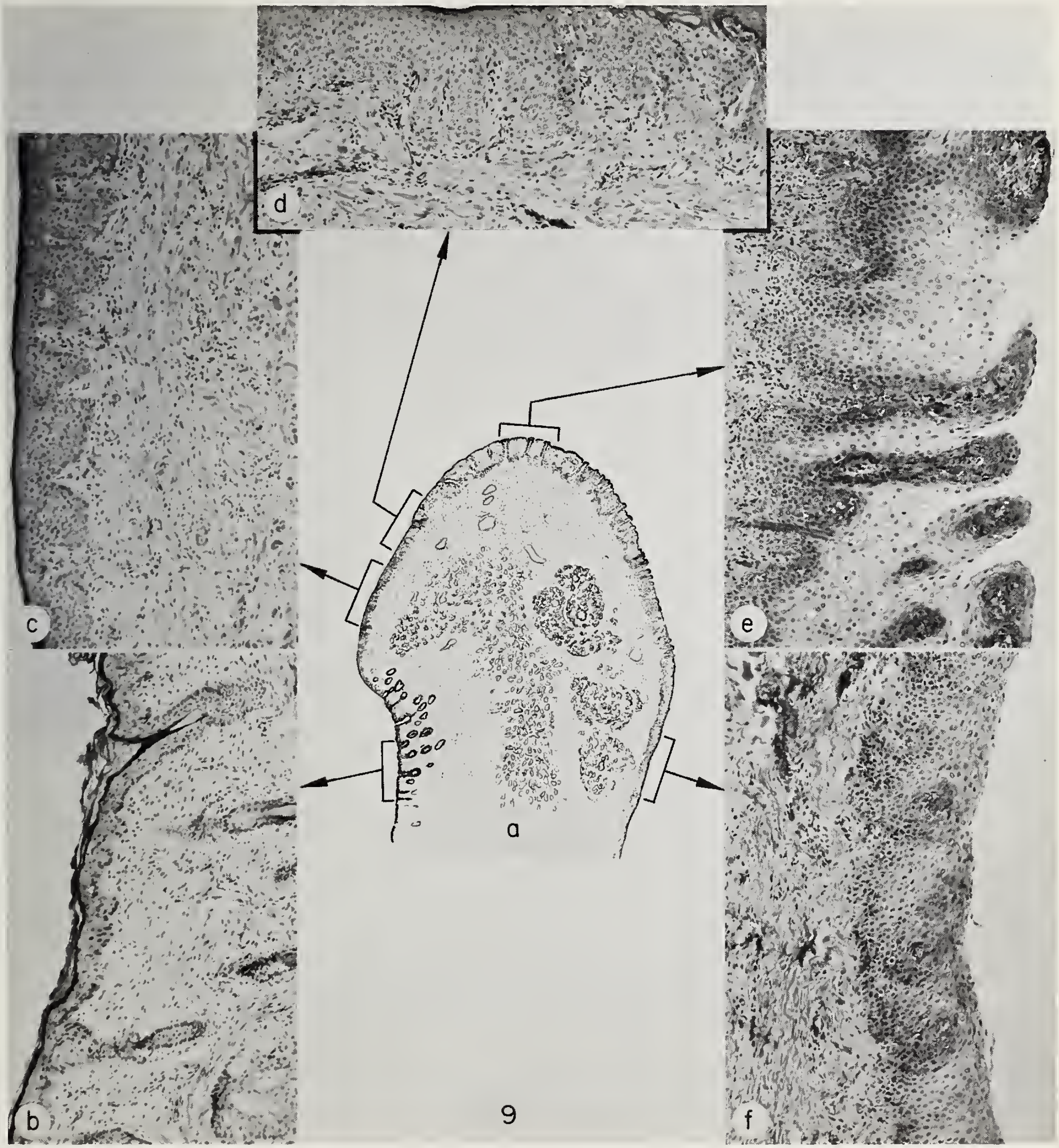

Figure 6–9. Histologic features of the integument from five areas of the lower lip of a 35 week infant (live-born).

(a) schematic drawing of section of the lower lip for reference to b through f.
(b) hairy cutaneous zone.
(c) glabrous zone.
(d) area of transition from keratinized to nonkeratinized epithelium, where glabrous and papillary zones meet.
(e) papillary zone.
(f) labial vestibular zone.

All H and E, x 140.

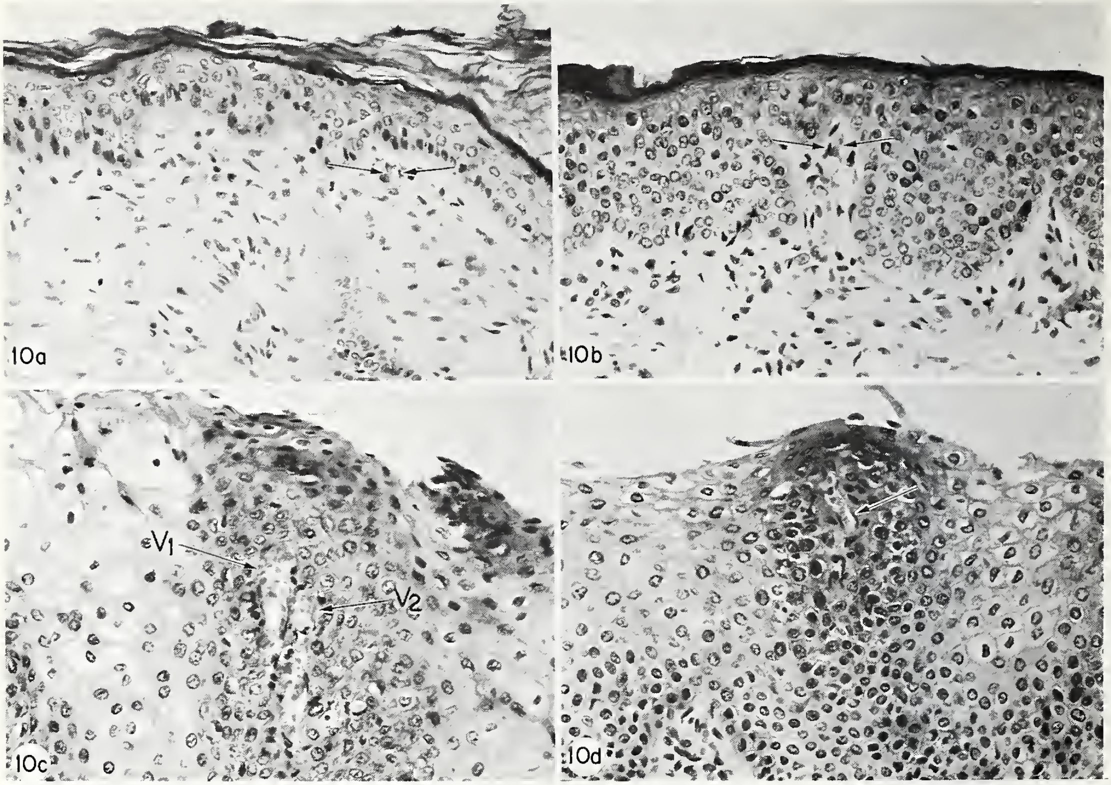

Figure. 6–10 Areas of the lip shown in Figure 6–9 at higher magnification, x 350. From the lower lip of a 35 week live-born infant.

(a) Hairy cutaneous zone. Note relatively thick keratin, thin epithelium, irregularities in the basement membrane (which are not true papillae) and infrequent small vessels (arrow).

(b) Glabrous zone. Note keratin and short mucosal papillae with small vessel (arrow).

(c) Upper half of a long connective tissue papilla in the papillary zone. Note absence of keratin, vacuolization of epithelial cells between papillae and both limbs (V1 and V2) of the large vessel within the papilla.

(d) Vestibular zone. Note smaller vacuoles within the epithelial cells and relatively small vessel (arrow) within short papilla.

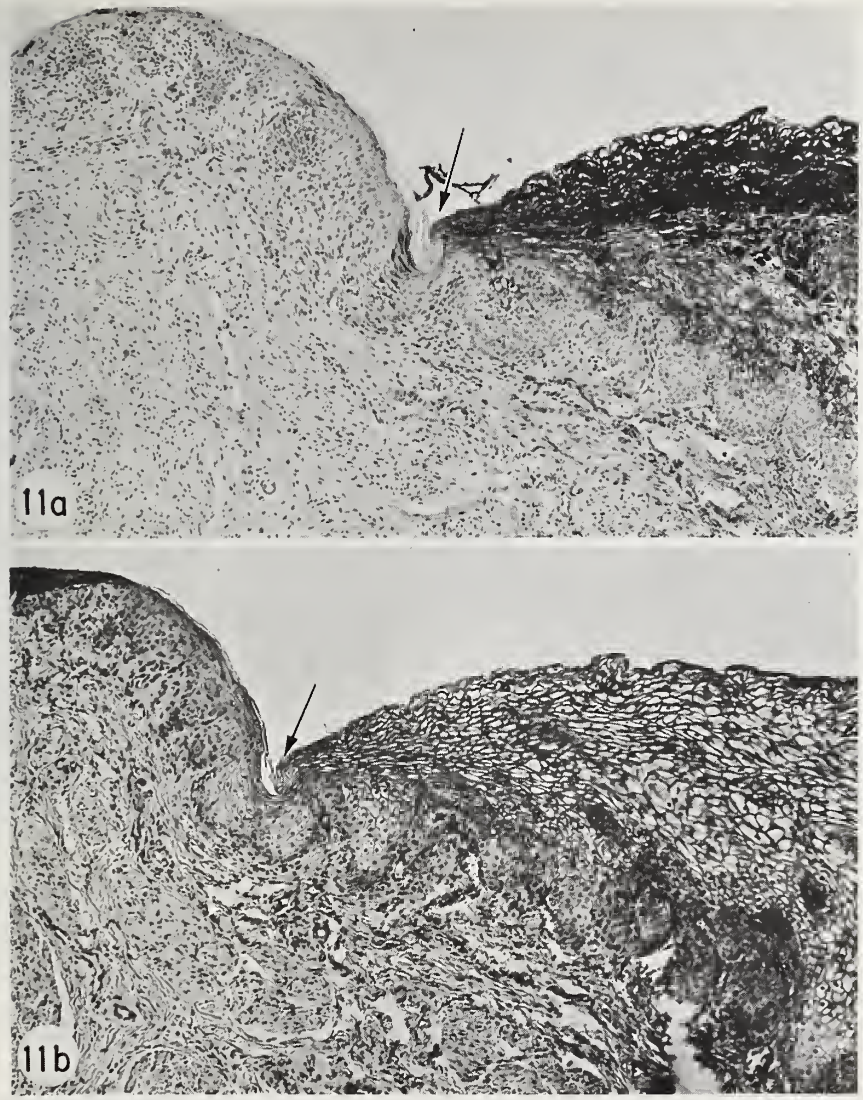

Figure 6–11. Lower lip in area of groove (arrow) between glabrous and papillary zones from a 29.5 week fetus (live-born). Figure 6–11a has been stained with PAS. Figure 6–11b has been stained with PAS after diastase digestion. x 88.

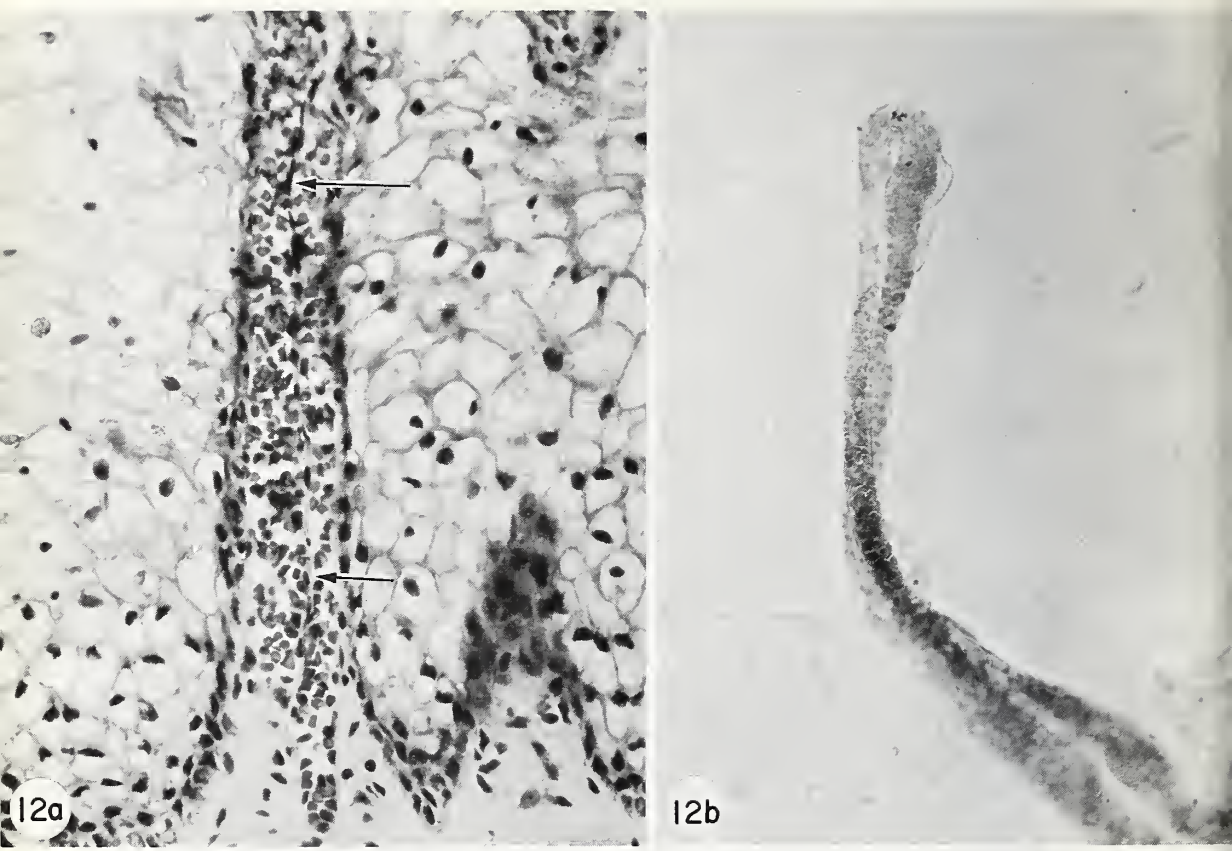

Figure 12. a) Adjacent limbs of a vascular loop, separated by thin walls (arrows), in a papilla from the upper lip papillary zone, of a 27.5 week stillborn fetus. H and E, x 350. b) Single vessel loop from papilla in the papillary zone of the upper lip, from a 37 week stillborn fetus. Vessel lumen is filled with erythrocytes. Surrounding epithelial cells have been teased away. Specimen embeded in Canada balsam, unstained, x 350.

larger at the base of the papilla and narrows gradually toward the tip. The average diameter is 20–35 microns at the base and 12–15 microns at the tip. Occasionally, extremely large papillary vessels are seen (50 microns diameter). These loops connect directly with large subpapillary vessels.

In older (36–40 week) fetuses, nerve fibers which enter papillae are larger in diameter than those seen in papillae of younger (26 weeks) fetuses. Large nerve fibers are seen at the base of each papilla, and accompany the vessels into it. Some small nerves appear to terminate close to the basement membrane at the base of the papilla (Fig. 6–14). Others follow the vessels upward, where they are lost in the darkstaining reticular fibers of the vessel wall (Fig.

Figure 6–14. Single nerve fibers (arrows) terminate close to basement membrane in an area between papilla in the papillary zone. Lower lip of stillborn fetus (40.5 weeks). Bodian, x 880.

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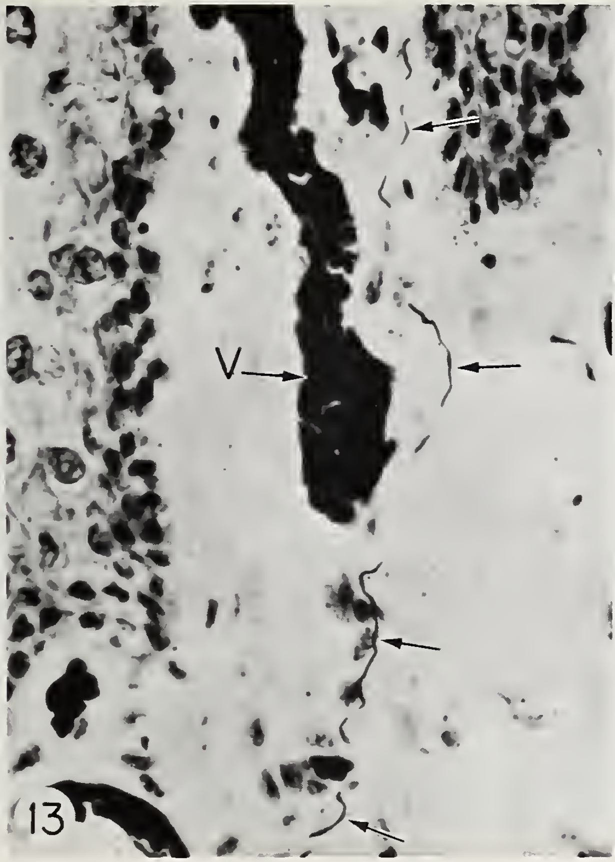
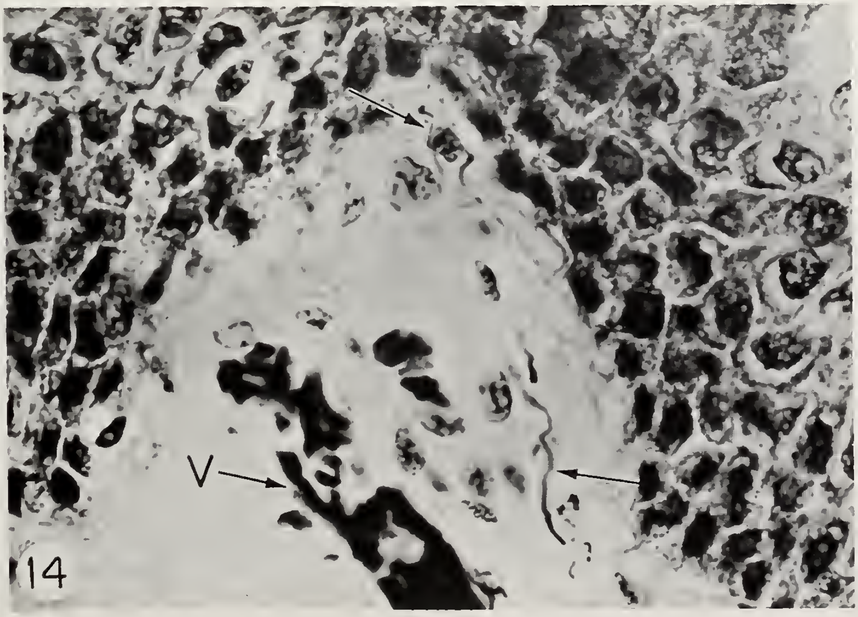


Figure 6–13. Papillary zone of the lower lip of a term (40.5 weeks) stillborn fetus. Single nerve fiber (arrow) and papillary vessel (V) entering papilla. Bodian, x 560.



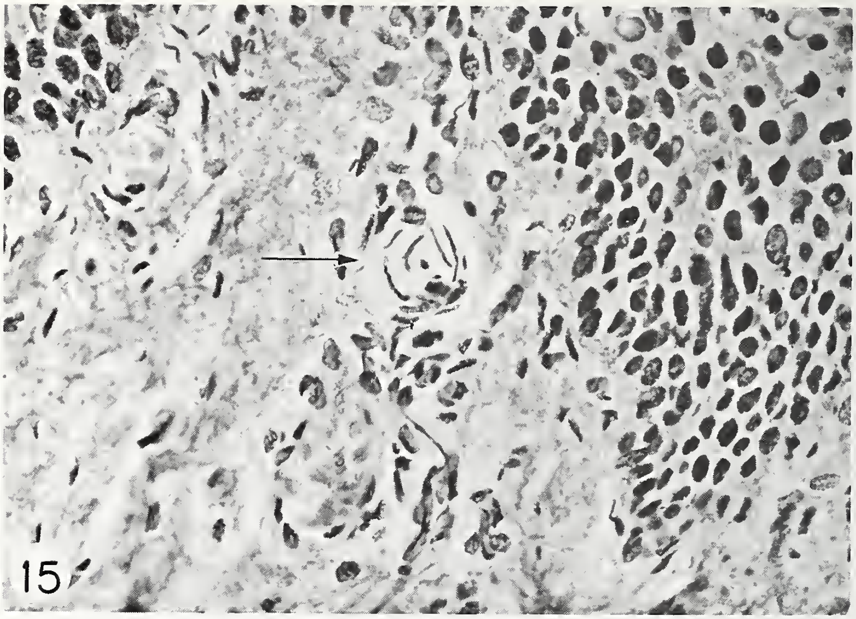


Figure 6–15. Complex nerve ending consisting of coiled nerve fibers surrounded by a clear zone. Papillary zone of lower lip of a term (41 week) live-born infant. Bodian, x 560.

6–13). In term fetuses, single or branching nerve fibers are found in the papilla tip, apparently terminating close to the basement membrane. Occasionally, in the term fetus, one sees complex endings with one or more large nerve fibers coiled about to form a spherical structure surrounded by a clear zone with faint concentric lines indicating connective tissue lamellae (Fig. 6–15).

About half way down the mucosal aspect of the lip, the epithelium becomes thinner (100 to 200 microns) (Fig. 6–9f). The transition is gradual. The epithelial cells have smaller vacuoles and correspondingly less glycogen. The papillae are shorter (150 microns). The vascular loops are also smaller (10 microns in diameter) (Fig. 6–10d). In the Negro fetus, pigment containing cells are less numerous. Single nerve fibers approach the epithelium but are less numerous.

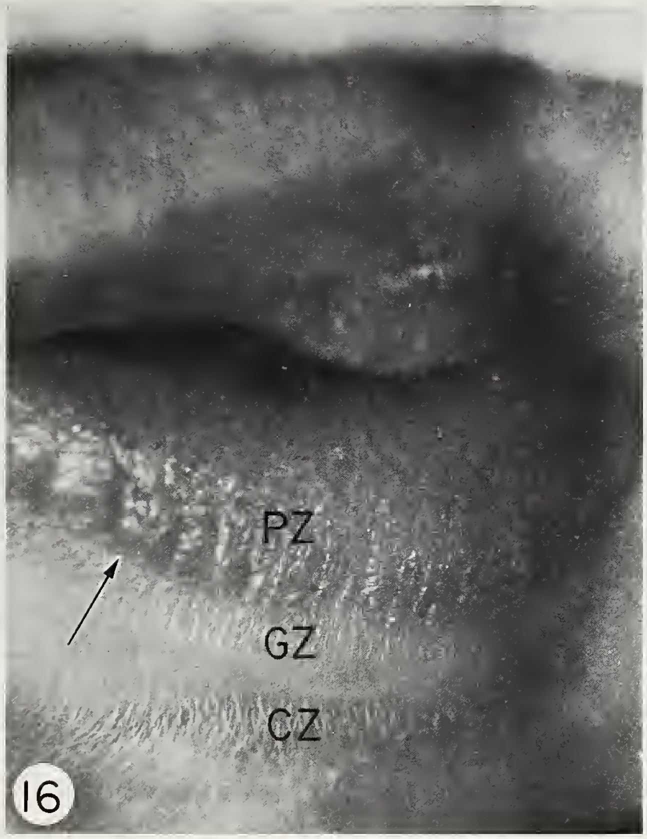


Figure 6–16. Lips of living term infant. Hairy cutaneous zones (CZ), glabrous zone (GZ) and papillary zone (PZ) are easily distinguished. Arrow points to groove between glabrous and papillary zones. x 3.5.

THE LIP IN LIVING NEWBORN INFANTS

Much can be learned by examining the lips of living infants*. The hairy cutaneous, glabrous and papillary zones are easily identified one from the other (Fig. 6–16). The mucosa of the vestibular zone also appears different from that of the papillary zone. A shallow groove is usually present, dividing glabrous from papillary zones. The papillary zone frequently appears flushed in term infants. In Negro infants, the papillary zone is usually more highly pigmented than the glabrous and vestibular zones and is frequently darker than facial skin. This is especially true in the premature infant where pigmentation in the papillary zone may increase during the first few weeks of life (Fig. 6–18).

In most infants, the tips of individual papillae have a milky color and project slightly above the surface of the lip giving the whole surface a granular appearance. There are 12 to 15 papillae per square millimeter in term infants. The

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*The lip area in 24 premature (<38 weeks gestation) and 30 term infants was examined. Twenty five term infants were studied with a surgical microscope with variable magnification up to 40x. Details of the vasculature were more clearly observed when a light coat of mineral oil was applied to the lip and skin.

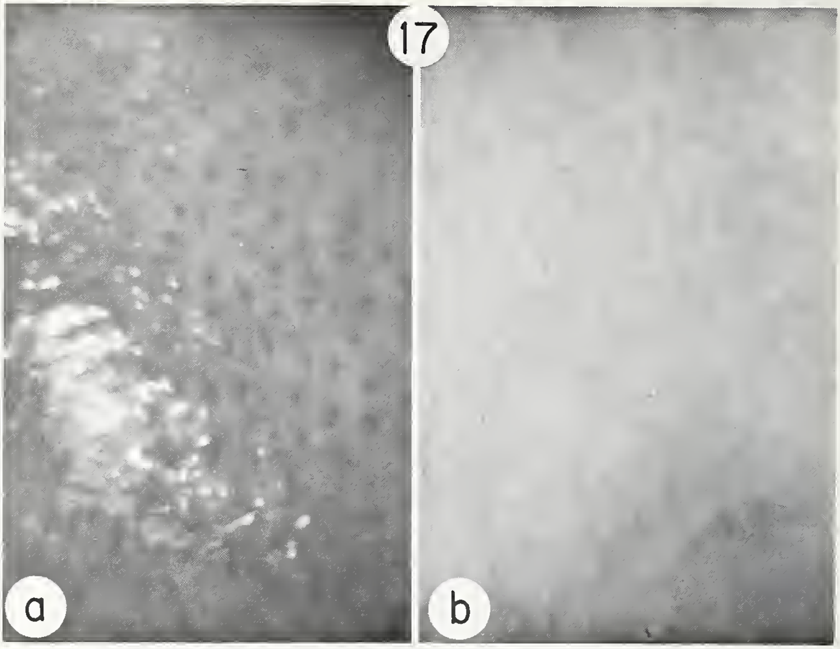

Figure 6–17. Surface of the lip in a living newborn infant.
(a) Papillary zone. Tips of papillary vessels beneath the surface appear as dark dots.
(b) Labial vestibular zone. Vessels beneath the surface have a reticular pattern.

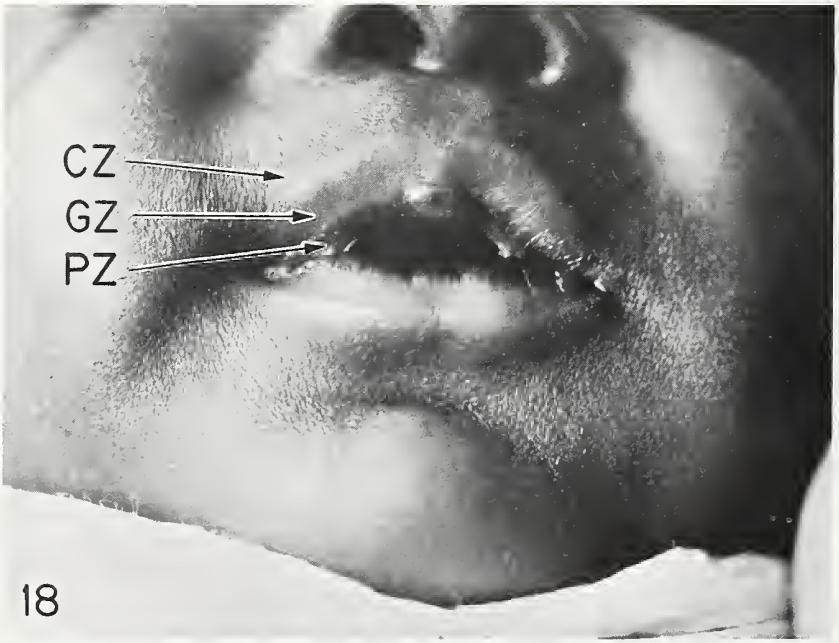

Figure 6–18. Mouth of a living premature Negro infant. The hairy cutaneous (CZ) glabrous (CZ), and darkly pigmented papillary zones (PZ) are easily distinguished.

epithelium of the papillary zone is both mobile and adherent. It sticks to the tip of a fine nylon filament. This adhesive force between the filament and the epithelial surface is sufficient to lift the tissue to form a peak or dome when the filament is withdrawn. This phenomenon does not occur when the filament is tested on the superior or inferior surface of the tongue or on the vestibular or glabrous zone surfaces.

One can see differences in the superficial vessels of the zones. Small red dots are visible to the naked eye in the papillary zone of most term gestation infants. These are, however, usually not apparent in small premature infants. Under 25 x magnification, these dots are identified as individual vascular loops projecting up through the translucent mucosa (Fig. 6–17a). The glabrous zone and the labial vestibular zone exhibit a reticular network of vessels beneath the integument rather than vessel loops (Fig. 6–17b). Within the first few days of life, vessel loops that have become obscured by a red or brown localized stain are frequently observed. These appear to be minute hemorrhages. By the third day of life, a light-colored epithelial scale or crusting frequently appears at the groove dividing the glabrous zone from the papillary zone. Under magnification these appear to be dry epithelial sheets that have begun to peel off. In some cases the whole papillary zone is involved. The glabrous zone and hair bearing zones remain relatively unaffected (Fig. 6–19). This phenomenon was noted in about half of the term infants, but less frequently and to lesser extent in premature infants.

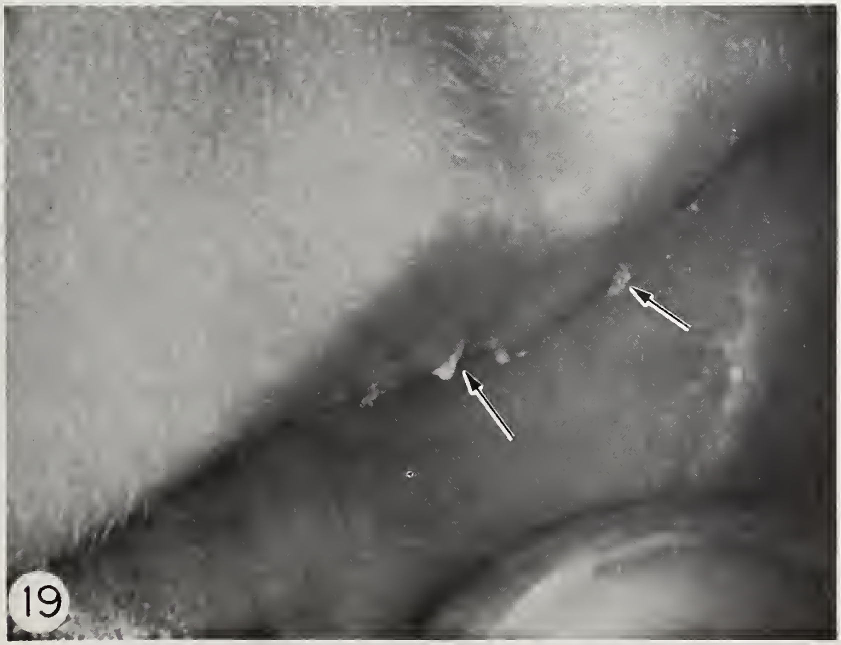

Figure 6–19. Crusts of epithelium (arrows) forming at the boundary between glabrous and papillary zones in a 4 day old term living infant.

POSTNATAL DEVELOPMENT

Some time after birth, the external features that distinguished the labial zones are obscured. This seems to be the basis for the belief that the "pars villosa" disappears after infancy.[24,27] In the Negro, changes in pigment distribution in the lip may be marked. In Negro adults, the outer lip is darker and tends to become lighter towards the line of approximation between upper and lower lips. Nevertheless, there are certain individual adults (Caucasian and Negro) that have a shallow transverse groove in the lip surface about 3 to 7 mm from the hair bearing cutaneous margin. This is similar to the groove between glabrous and papillary zones in the infant. Histologic descriptions of the adult lip are incomplete. Anatomical sources describe short connective tissue papillae in the vermilion border of the lip near the cutaneous margin. These are said to be replaced by longer papillae toward the line of approximation between the upper and lower lip.[27,28] Published low power photomicrographs[23,29] of sagittal sections of lip, presumably from adult subjects* seem to confirm this. Since this transition from short to long papillae is said to take place at the line of approximation between upper and lower lips[27] rather than on the externally visible portion of the lips (as in the infant), the relation of the distribution of papilla in the adult to the fetal labial zones is unclear.

Binnie and Lehner[4] examined the histology of the lip at the angle of the mouth. They classified tissue zones according to epithelial thickness. They found three zones in infants: (1) a hair bearing cutaneous zone, (2) a glabrous zone with abrupt transition to (3) an oral mucosal zone. The same zones were described in adults with the exception of a fourth zone found between the glabrous zone and the oral mucosal zone. This fourth zone had an epithelial depth intermediate between the two. Papillae, glycogen and vasculature were not described.

There do not appear to have been any systematic histologic studies of sagittal sections of the adult lip other than at the angle of the mouth. We have looked at several tissue samples from the appositional margin of the lips of older children. The connective tissue papillae are long and well developed. The surface epithelium is parakeratinized. Epithelial cells are not vaculoated and do not have large amounts of glycogen. There is a small amount of glycogen in cells between the basal and surface layers. The connective tissue papillae are a good deal wider than in infants. Organized nerve endings can be found near the tips of papillae (Fig. 6–21). Organized nerve endings of the lamina propria are surrounded by connective tissue laminae and seem to be more numerous and larger than in the infant (Fig. 6–20). As we have not studied whole lip sections, we can say little about the zonal characteristics of the epithelium.

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*No age given by either source.

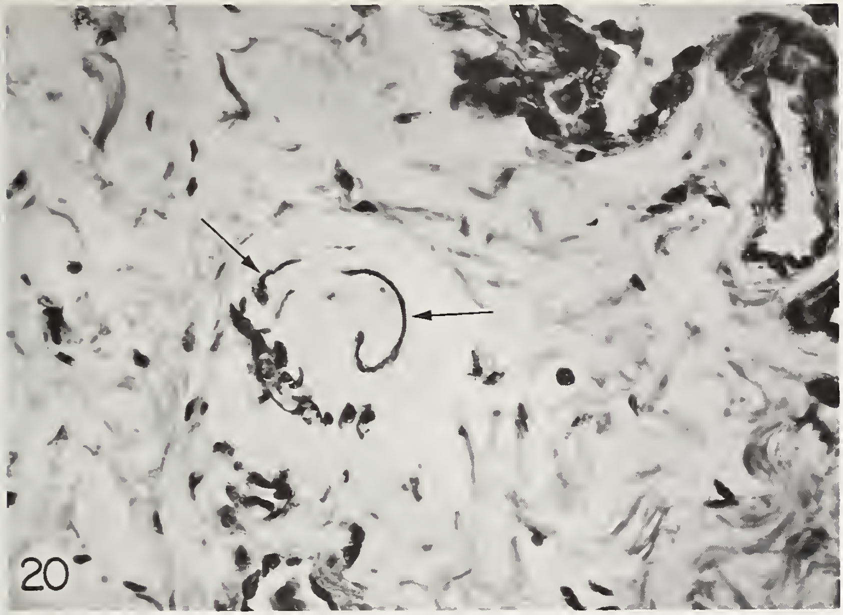

Figure 6–20. Complex nerve ending from the appositional margin of the lower lip of a 7 year old child. Large fibers (arrows) are surrounded by connective tissue lamellae. Bodian, x 560.

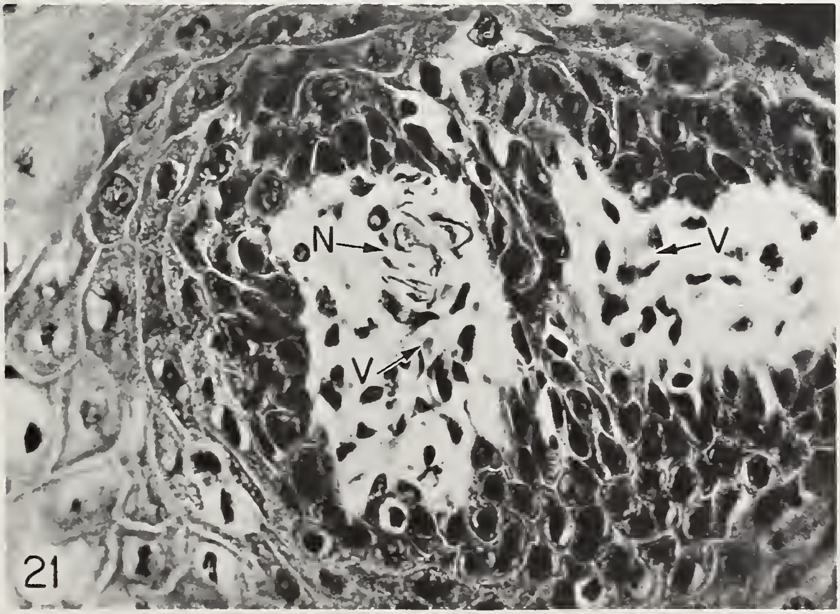

Figure 6–21. Complex nerve ending (arrow labelled N) from the appositional margin of the lower lip of a 7 year old child. It is composed of relatively small fibers coiled near the tip of a connective tissue papilla. Note relatively small size of papillary vessels (arrows labelled V). From the same source as in Fig. 6–20. Bodian, x 560.

DISCUSSION AND A COMMENT ON FUNCTION

The structure of the lip integument in the infant can be summarized as follows. In late fetal life it consists of four structurally different tissue types. Each is characterized by specific differences in degree of keratinization, morphology of superficial cells, pigmentation, size of connective tissue papillae, vascularization and innervation. The transition between the different epithelial types tends to be abrupt. From the standpoint of zonal arrangement, these findings do not differ markedly from previous descriptions and are in general agreement with more recent studies pertaining to fetal lip vascularization[7] and innervation.[28,32,34]

How can we explain this precise arrangement of tissues which later becomes obscured? The variable features of the infant labial zones, such as thickness of the epithelium, papilla length, and degree of keratin formation, are general features which vary from region to region throughout the oral cavity. The specificity of epithelial type is thought to be, by and large, genetically predetermined.[13] However, tissue of one type may become altered when exposed to chronic trauma or inflammation and take on new characteristics. For example, it may change from nonkeratinized to keratinized mucosa.[12,13] Therefore, the transformation one sees in fetal lip epithelium could be either a functional adaptation of the integument in response to environmental stimuli or the expression of a genetically determined pattern. It would appear that the second of these possibilities is most likely, since formation of zones occurs early in fetal development, and since factors which might induce changes such as trauma, dehydration or thermal gradients, are minimal *in utero*. On the other hand, since fetal oral activity also develops early,[16] mechanical stimuli resulting from lip-lip, hand-lip and tongue-lip contacts cannot be entirely ruled out as influences on epithelial development.

The disappearance of the gross features distinguishing the labial zones in the adult is more difficult to explain. It is possible that the fetal zones are purely embryonic remnants that become transformed with maturation, analogous to the fetal lobulations of the kidney or the ostium secundum of the heart. On the other hand, it may well be that the zones are only superficially obliterated by functional adaptation of the epithelium, while the basic underlying zonal pattern remains.

Specific structures imply specific functions. In order to associate a function with a given structure, one must be able to objectively demonstrate a property that is specific to it. Second, one should be able to prove what is implied; namely, that removal of this property is a disadvantage to the individual. While engaging in such exercises it is well to remember that a structure may have more than one function. On the lips of the newborn infant, the tall papillae with prominent nerves and large vascular loops hold our attention. As noted earlier these papillae are not strictly limited to the lips but extend on to the buccal mucosa. In the adult, the deep buccal epithelium with long papillae adjacent to the molar occlusal surface is thought to have a protective role in an area where trauma is frequent.[25] Likewise, the papillary zone of the infant lip appears to have a mechanical role. It is adhesive or "sticky" — more so than epithelium of the glabrous or vestibular zones. As such, it should have an advantage for the infant in maintaining oral seal during suckle feeding. In the infant, this seal between lip and nipple keeps fluid in and air out. Inability to maintain oral seal presumably accounts for some of the feeding difficulties of infants with cleft lips (and intact palates). Difficulties with bottle or breast feeding are said to result from both inability to generate intraoral negative pressure and from swallowing of air during feedings. Feeding is improved by surgical repair or a prosthesis which covers the lip defect.[10] Air seal at the lips also helps the infant hold onto the nipple. Nursing mothers who have difficulty removing an infant from the breast are advised to break this seal by inserting a finger in the corner of the infant's mouth.[3]

Might this area of the lip have other special properties? An interesting observation of possible relevance is that, in adults the vermilion border of the lip is not uniformly sensitive. Grossman found a three fold difference in light touch sensitivity between a site several millimeters from the cutaneous margin and a site in the labial appositional line.[14] There are no similar studies available for infants. It is of interest that organized nerve endings are relatively infrequent in the lip of the newborn. Organized corpuscular nerve endings in the finger (Meisner corpuscles)[9] and in the tongue of human and monkey neonates also are less developed than in the adult.[17,20] Complex endings such as these are presumed to be specific mechanoreceptors. On the other hand, it is well to remember that one need not have complex corpuscular endings in order to have a low threshold for touch or thermal stimuli. The cornea, more sensitive than the finger tip to light touch, has only simple nerve endings.[18] Perhaps the most sensitive thermal receptor known (the pit organ of pit vipers) consists of numerous relatively simple nerve endings within a thin epithelial membrane.[6] It has often been stated that the relation of nerve endings to nonneuronal supportive epithelial structures may markedly influence sensitivity.[8] Such is the case of vibrissae which amplify mechanical input, transmitting movement to nerve fibers about the hair sheath. It is conceivable that the arrangement of nerves to looped vessels and papillae of the papillary zone could have a modifying effect on sensory reception by the infant lip. While the latter lies within the realm of broad speculation at present, extended research in this area would seem to be warranted.

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Chapter 7

NON-NUTRITIVE SUCKING IN GREAT APE AND HUMAN NEWBORNS: SOME PHYLOGENETIC AND ONTOGENETIC CHARACTERISTICS

JOSEPHINE V. BROWN

This paper presents two studies in which non-nutritive sucking is compared across newborns of three different species and across human newborns at two levels of gestational age. The studies focus on the pattern of non-nutritive sucking and the influence of external auditory and visual stimuli on this response. The question to be asked is: to what extent can a response in one modality (i.e., non-nutritive sucking) function as an index response for the functioning of another modality?

In the human newborn, investigators have regarded non-nutritive sucking as a useful dependent variable in the study of sensory capacities.[11] Sensory capacities have often been investigated with a distraction paradigm, in which acoustic or visual stimuli are presented to infants engaged in non-nutritive sucking. With this paradigm, Bronshtein et al.[2] and Keen[13] found suppression of sucking after the onset of a tone which they used as an indication of the infant's auditory responsiveness. Other investigators[12,10] have been unable to demonstrate this suppression and have most often concluded that an apparent lack of sucking suppression to an external stimulus is due to problems with the interpretation of the sucking record. Since non-nutritive sucking of human newborns occurs in regular alternations of bursts and pauses in the absence of any apparent external stimuli, it is difficult to determine whether a cessation in sucking is associated with an external stimulus or is simply a regular pause.[8,17,18]

A failure to obtain sucking suppression, however, might also be due to other factors. When an external stimulus is superimposed on ongoing non-nutritive sucking, the infant has to deal with two inputs. A response to the external stimulus might occur only if the sensory input from this stimulus is stronger than the intraoral stimulation obtained from the nipple. Cairns[5] tested this hypothesis by comparing sucking suppression to an auditory stimulus of infants sucking on either a regular nipple or a tube. Although the tube and the reg-

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ular nipple elicited different sucking rates, suppression was not differentially affected.

There is another, and perhaps more plausible, reason for the infant's failure to suppress sucking in response to an external stimulus. In a distraction procedure, an infant's response to an external stimulus is indicated by an alteration of his sucking pattern. If the infant does not alter his sucking behavior, we do not know if this is due to a failure on the part of the subject to process the external stimulus, to suppress his sucking, or both. The logic behind the distraction paradigm rests on the assumption that sucking and auditory or visual behavior are differentiated, i.e., are behaviors that can change independently of each other, such that changes in the first behavior can be taken as an index of the level of functioning of the second. The possibility exists, however, that both of these kinds of behaviors vary together and are both a reflection of the same underlying CNS organization or similar organismic variables.

This paper reports two studies. In the first study the non-nutritive sucking pattern and the effects of external stimuli on ongoing non-nutritive sucking was compared in subjects of three species: humans, chimpanzees (*Pan*), and orang-utans (*Pongo*). These species are closely related, yet their non-nutritive sucking patterns are controlled by qualitatively different CNS's. Wolff[21] found that infant mammals showed a continuous non-nutritive sucking pattern rather than the pattern of rhythmical alternations of bursts and pauses so characteristic of the human infant. On the basis of these findings, he suggested that "differences between humans and other mammals in the temporal organization of sucking behavior may represent a qualitative change in central nervous system control over the sucking reflex. . . ." (p. 363).

The second study was similar to the first one except that the subjects were human newborns at two different estimated gestational levels. It has been shown that the temporal pattern of sucking of the human newborn is affected by various organismic variables. For instance, the burst length is slightly longer, the sucking rate is higher and the variability is lower during regular sleep than during irregular sleep.[22] Parmelee[15] states that "premature infants have very irregular periods of sleep and wakefulness and that well-defined states are not sustained." (p. 352). He suggests that "the maturation of the ability to sustain sleep and wakefulness coincides with the development of sustained visual attention." (p. 352). One could postulate, therefore, that as the infant matures the influence of the underlying organismic variables (i.e., state) on sucking behavior and responsiveness to external stimuli decreases.

PHYLOGENETIC COMPARISONS OF NON-NUTRITIVE SUCKING AND SUCKING DISTRACTIBILITY

Reports in the literature indicate that non-nutritive sucking occurs in newborn orang-utans[23] and chimpanzees.[9] Wolff[21] recorded both nutritive and non-nutritive sucking of a variety of mammals including an 8-month-old gorilla (*Gorilla*) and a 15-month-old chimpanzee. The great ape infants (as other mammals) sucked continuously both nutritively and non-nutritively. Continuous non-nutritive sucking in beagle puppies has also been reported.[19] The rhythmical pattern of bursts and pauses so characteristic of non-nutritive sucking in human infants seems to be confined to the human species.

The following study was designed to: (a) describe the development of non-nutritive sucking in human, chimpanzee, and orang-utan newborn infants; and (b) evaluate the distractibility of this response across the three species.

Subjects

The human subjects were five black male and five black female newborns. All infants were considered full term and normal and weighed between 2650 and 3660 gm (mean 3185.5 gm) at birth. They had Apgar[1] ratings of 9–10, five minutes after birth. The mothers of the infants had received less than 100 mg analgesia between one and five hours prior to birth*. Only three of the mothers had received general anesthesia.

The great ape subjects were four chimpanzee and two orang-utan newborns, separated from their mothers shortly after birth and with no nursing experience. The chimpanzee sample consisted of one male, born at term, one female, born slightly premature, and a set of female twins born by caesarian section. The infants weighed between 1280 and 1800 gm (mean 1527 gm) at birth. The orang-utan sample consisted of one male born at term, weighing 1940 gm, and one female born approximately one month premature, weighing 1600 gm.

Procedure

Non-nutritive sucking was recorded from all subjects between two and seven hours after birth, before the infants had any experience with nutritive sucking and during 24-hour intervals thereafter. Subsequent recordings from human infants were made on Day 2 through Day 4 prior to the 7 a.m. feeding, and from chimpanzee and orang-utan infants on Day 2 through Day 5 prior to the 5 p.m. feeding. Each recording session was 10 minutes long.

On Day 3 for human and Day 4 for great ape infants, an auditory stimulus, a loud bell lasting five seconds, was presented five times while the infants were sucking. Presentations were uniformly made after the first two sucks of the first burst that occurred in the second, fourth, sixth, eighth, and tenth minute of the session.

Apparatus

Non-nutritive sucking was recorded with a standard size Davol nipple. A small plastic disc with a 4.8 mm hole in the middle was fitted inside the wide portion of the nipple and used to couple the nipple to a piece of surgical tubing, 50.8 cm long and 7.9 mm in diameter. This tubing was attached to a Sanborn Model 267 AC differential pressure transducer. Analog recordings of sucking pressure were made on a Sanborn polygraph at a paper speed of 5 mm per second. Figure 7–1 illustrates the experimental set-up with a chimpanzee subject.

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*Subjects had been preselected on this criterion. The most common analgesics used during labor were a combination of 25 or 50 mg of Demerol and 25 mg Phenergan (Promethazine), or a combination of 25 mg Demerol and 10 or 20 mg Largon (Propiomazine).

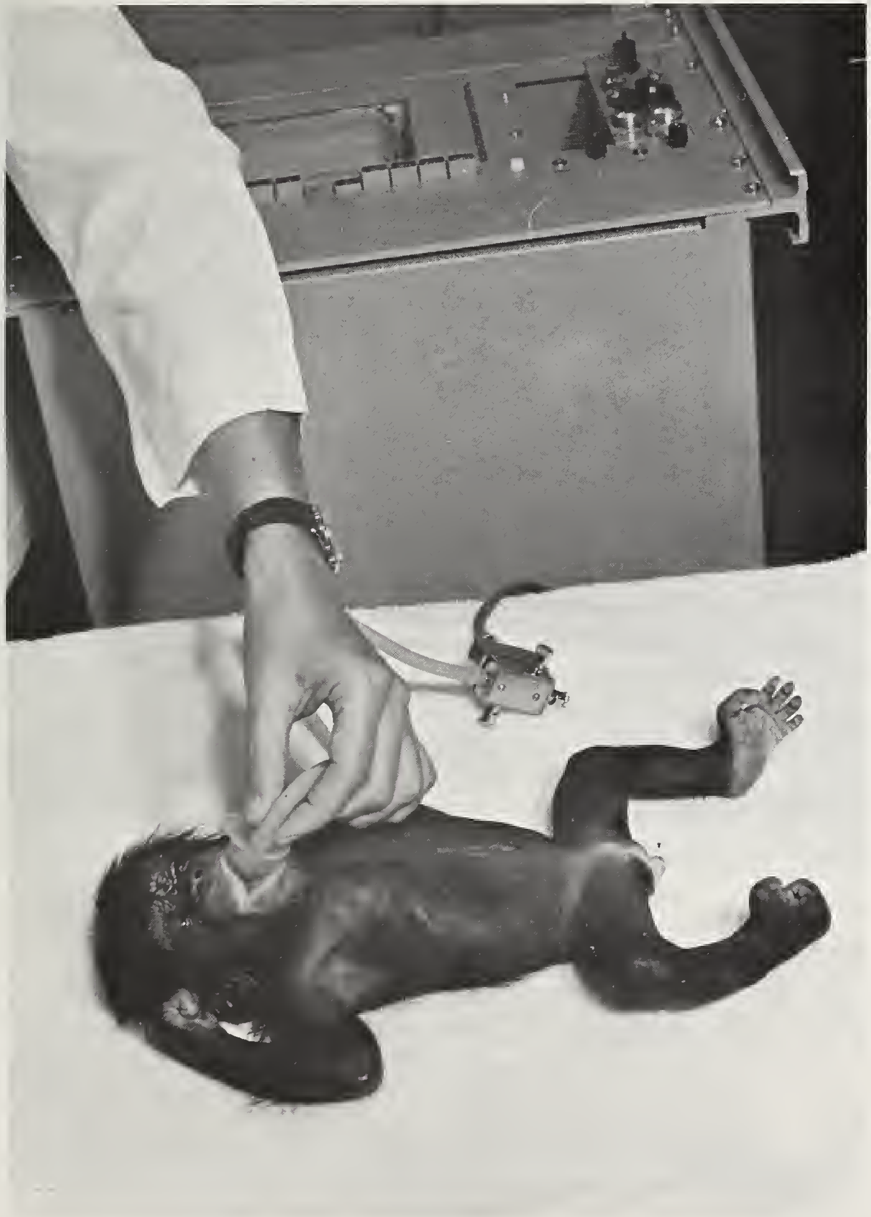

Figure 7–1. An illustration of the manner in which non-nutritive sucking was recorded. The subject is a newborn chimpanzee.

The definition of a suck was based on two criteria: (1) amplitude, a pen deflection of 2 mm or more from base (corresponding to 10 mm Hg negative pressure); and (2) steepness of peak, a 2 mm pen deflection at the peak over 1 mm movement of the paper (0.2 sec) and a 2 mm return from peak over 2 mm movement of the paper (0.4 sec). These criteria had been previously established.[11]

Dependent variables

The following measures were analyzed for each ten-minute record: percentage of time spent sucking; total number of sucks; total number of sucking episodes (two or more sucks occurring less than two seconds apart were considered as belonging to the same episode); mean number of sucks per episode; rate of sucking during an episode; mean duration of intervals between episodes (IBI) in seconds.

Results

All sucking measures were analyzed separately using analyses of variance. Sex and days were the factors analyzed for the human subjects, and days was the factor analyzed for the great ape subjects.

Table 7–1 summarizes the changes in sucking behavior from Day 1 to Day 4 for human subjects and from Day 1 to Day 5 for chimpanzee and orang-utan subjects.

The rate while sucking ($F\ 2,16 = 3.80$, $p < .05$), time spent sucking ($F\ 2,16 = 5.95$, $p < .05$), and number of sucks per ten-minute testing session increased significantly over days for the human subjects ($F\ 2,16 = 14.24$, $p < .001$).

The chimpanzee infants also spent more time sucking ($F\ 3,9 = 13.53$, $p < .001$) and made more sucks ($F\ 3,9 = 9.25$, $p < .005$) over days. The data from the two orang-utan subjects, although not statistically significant, were generally consistent with those of the newborns of the other species.

Most interesting, however, are the differences between the three species in the changes of their sucking pattern over the first few days of life. The pattern for humans changed very little over days, while chimpanzees and orang-utans sucked in an unpredictable pattern on Day 1 and gradually shifted to continuous sucking at approximately Day 5.

TABLE 7–1

MEAN CHANGES IN SUCKING OVER DAYS

	HUMANS *Day 4—Day 1*	*CHIMPANZEES* *Day 5—Day 1*	*ORANGUTANS* *Day 5—Day 1*
Percent of time spent sucking	+ 14[2]	+ 61[1]	+ 29[1]
Total number of sucks	+202[1]	+468[1]	+151[1]
Rate in sucks/sec.	+ .2[2]	+ .3[1]	0
Number of sucks/sucking episode	+ 1	+100[1]	+246[1]
Number of sucking episodes	+ 10[3]	+ 4	− 20[1]
Duration of IBI in sec.	− 2[2]	− 19[1]	− 5[1]

[1] All subjects changed in same direction
[2] P<.05
[3] P<.10 (one subject out of 10 reversed)

Table 7–2 summarizes the sucking measures of the three species on the last day of recording. Although no statistical comparisons could be made between them, there are some reliable and real differences (i.e., the species did not overlap on these measures). These differences are found in the rate of sucking with humans sucking at a rate of 1.8 sucks per second, chimpanzees at a rate of 1.3 sucks per second, and orang-utans at a rate of .9 sucks per second. More important differences, however, are found in the pattern of sucking as described by the number of sucks occurring in each sucking episode. Human infants sucked in an alternation of bursts and pauses, with each burst consisting of approximately 14 sucks. Chimpanzees and orang-utans, however, did not show such a pattern and tended to suck continuously. For example, one of the orang-utans sucked without stopping for the entire ten-minute period and made 530 sucks. These differences in sucking patterns are illustrated in Figure 7–2, which shows an analog recording of non-nutritive sucking of one subject of each of the three species.

Because of the difference in the sucking pattern between the three species, it was not possible to devise a comparable quantitative method of measuring sucking disruption. Therefore, six graduate students who were unfamiliar with research on sucking were asked to judge whether a disruption had occurred by comparing a ten-second period beginning with the onset of the bell with one 30-second period prior to and another immediately following this ten-second period. A disruption was defined as a decrease in rhythmicity only and was not to be judged on the basis of changes in amplitude. The results of this analysis showed that humans disrupted their sucking on 58 percent of the trials, chimpanzees on 40 percent of the trials, and orang-utans on 20 percent of the trials. The inter-observer agreement

TABLE 7–2

SUMMARY OF SUCKING MEASURES (MEANS AND STANDARD DEVIATIONS)

	HUMANS *Day 4*	*CHIMPANZEES* *Day 5*	*ORANGUTANS* *Day 5*
Percent of time spent sucking	61 ± 9	78 ± 13	99 ± .6
Total number of sucks	642 ± 103	572 ± 140	535 ± 55
Rate in sucks/sec.	1.8 ± .1	1.3 ± .1	.9 ± .3
Number of sucks/sucking episode	14 ± 4	107 ± 4	282 ± 48
Number of sucking episodes	49 ± 12	18 ± 14	1 ± .7
Duration of IBI in sec.	5 ± 2	10 ± 9	2 ± 0

ranged from 83 percent for humans to 97 percent for orang-utans. This seems to indicate that at least across species the likelihood of obtaining a disruption in sucking decreased as the subject sucked in fewer episodes.

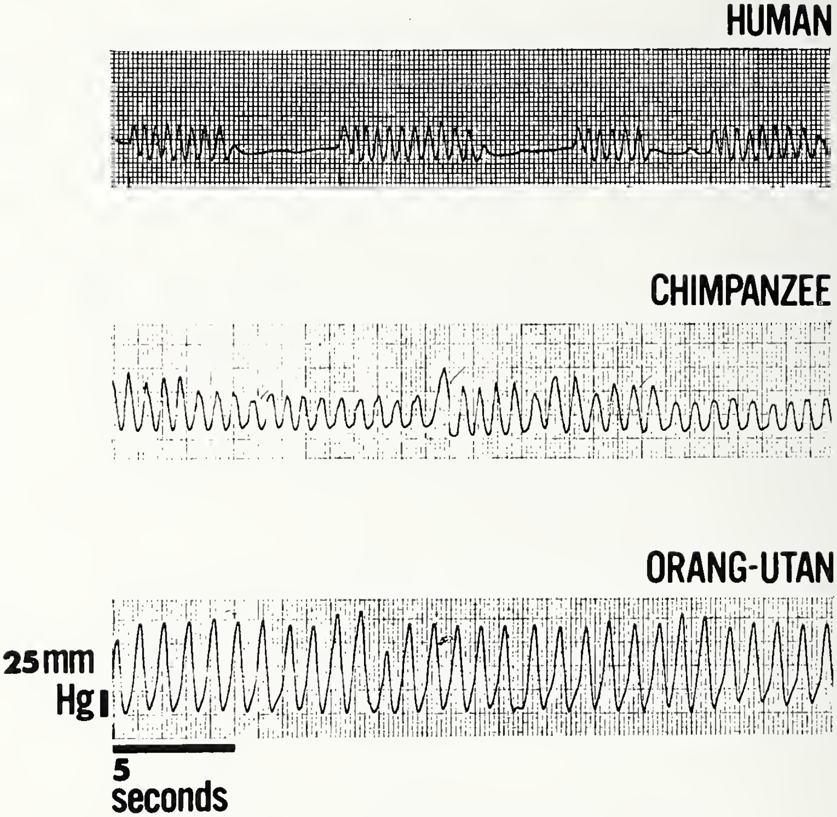

Figure 7–2. Analog records of negative non-nutritive sucking pressures of a newborn human, chimpanzee, and orang-utan subject.

ONTOGENETIC COMPARISONS OF NON-NUTRITIVE SUCKING AND SUCKING DISTRACTIBILITY

The second study deals with the possibility that qualitative changes in the sucking response may occur in human infants during their course of development and that these changes might be reflected in changes in response pattern and disruptibility. In this study, visual stimuli were added because visual responsiveness can be measured independently of sucking disruption, and it was hoped that this measure would reflect levels of maturity even if no changes in non-nutritive sucking were found. The auditory stimulus was identical to that of the first study. It was found that this stimulus, because of its loudness, maximized the possibility of obtaining a sucking disruption. The specific aim of the next study was to (a) compare the topography of non-nutritive sucking of infants of low estimated gestational age (LEGA) with that of infants born at term (HEGA), and to (b) investigate the disruptibility of the sucking response to auditory and visual stimuli.

Subjects

Ten black males and ten black females were selected randomly from each of two estimated gestational levels. The LEGA infants ranged between 33 and 36 weeks of gestation (mean 35.5 weeks) as defined by the obstetrician. The term infants were between 40 and 44 weeks of gestation (mean 42 weeks). All infants were considered normal. Although infants were selected only if they weighed more than 2250 gm, the weight of the LEGA infants turned out to be significantly lower than that of the term infants (F 1,36 = 13.96, $p < .001$). The weight of the LEGA infants ranged between 2270 gm and 3420 gm (mean 2851 gm) and that of the HEGA infants ranged between 2810 gm and 3890 gm (mean 3254 gm). Apgar and drug criteria were the same as those for the human subjects in the previous study.*

Procedure

All infants were tested prior to the 7 a.m. feeding. The baby was placed in an infant seat which was reclined at a 30° angle. After one minute of non-nutritive sucking, two visual stimuli were presented. The first stimulus consisted of three large black dots drawn on the diagonal of a sheet of white paper (24 x 27.5 cm), the second stimulus was a schematic face drawn on a similar size sheet. These particular stimuli were chosen because it had been shown in previous studies that they are potent stimuli and elicit differential attention in newborns.[20] Each stimulus was presented at a distance of 20 to 25 cm from the baby's face as follows: five seconds stationary, ten seconds movement in a vertical direction, five seconds stationary, and ten seconds of movement in a horizontal direction. There was an intertrial interval of 30 seconds between the presentation of the two visual stimuli. The infant had access to the nipple during the entire time. After the presentation of the visual stimuli, the baby was placed supine in a crib, and non-nutritive sucking was recorded for four minutes. During this recording, a loud bell was sounded for five seconds after the first two sucks of the first burst in Minute 2 and Minute 4.

Dependent variables

The sucking measures were similar to those in the previous study. The following auditory and visual measures were also analyzed: auditory suppression ratio (number of sucks during 10 seconds from the onset of the bell divided by the number of sucks during a 10-second period starting with the third suck in the previous burst plus number of sucks during 10 seconds from the onset of the bell); visual suppression ratio (number of sucks during the 30-second presentation of the visual stimulus divided by the number of sucks in the 30-second period prior to the presentation of the stimulus plus the number of sucks during the presentation of the visual stimulus); duration of visual behavior in seconds; quality of visual behavior as rated on a 10-point scale.[7] The suppression ratio has an expected value of < .5 if the infant decreases his sucking, of .5 if no change in sucking occurs, and of > .5 if the infant increases his rate of sucking to the stimulus. This measure is more sensitive in picking up increases or decreases in sucking than absolute difference scores, since it takes momentary changes in baseline sucking rates into consideration.

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*The analgesics most commonly used during labor were similar to those used with the subjects of Study I. There was no difference in the total amount of analgesics given to the mothers of LEGA and term infants.

Results

Sucking measures were analyzed separately with analyses of variance. The factors analyzed were EGA (estimated gestational age) and sex. No age or sex differences were found in any of the sucking measures. The sucking pattern of both groups of infants were comparable to those reported for the human subjects in the previous study and will not be further discussed.

The suppression ratios to the auditory and visual stimuli are summarized in Table 7–3. No age or sex differences were found in the amount of suppression. However, the sucking of infants of both age groups was significantly more suppressed on Trial 1 than on Trial 2. This was the case for both the auditory (F 1,36 = 4.13, $p < .05$) and the visual stimuli (F 1,36 = 3.51, $p < .10$). These findings suggest that habituation did occur over trials when sucking was taken as the dependent variable. Since suppression ratios to the auditory stimuli and the visual stimuli were calculated differently, no comparison could be made in the amount of suppression to stimuli presented in the two different modalities. Furthermore, the stimuli themselves were not comparable, since they differed in levels of intensity.

The term infants and LEGA infants differed significantly in their visual behavior. Term infants looked more at the visual stimuli than LEGA infants (F 1,36 = 16.35, $p < .001$) and obtained a significantly higher score on the visual scale (F 1,36 = 15.14, $p < .001$). The mean visual scores obtained by the HEGA infants in this study was nearly identical to that obtained by black infants in Graham's study.[7] There was no significant trend over trials in the

TABLE 7–3

SUMMARY OF MEASURES OF AUDITORY AND VISUAL BEHAVIOR OF HUMAN NEWBORNS (MEANS AND STANDARD DEVIATIONS)

	Low EGA		*High EGA*	
	Trial 1	*Trial 2*	*Trial 1*	*Trial 2*
Sucking suppression ratio to bell	.39 ± .14	.47 ± .11	.39 ± .13	.43 ± .21
Sucking suppression ratio to visual stimulus	.48 ± .08	.52 ± .11	.46 ± .10	.50 ± .08
Time spent looking in sec.	6.7 ± 5.7	6.9 ± 6.5	15.6 ± 6.2	14.0 ± 7.2
Visual score	4.4 ± 2.7	4.1 ± 3.0	7.3 ± 2.3	7.6 ± 2.4

amount and quality of visual behavior in the group of LEGA or the group of term infants.

Figures 7–3 and 7–4 illustrate the fact that the changes in behavior over trials are different when sucking is used as the dependent variable than when direct visual measures are used. They show that sucking suppression to visual stimuli recovered over trials in both groups of infants while the amount of time spent looking remained the same.

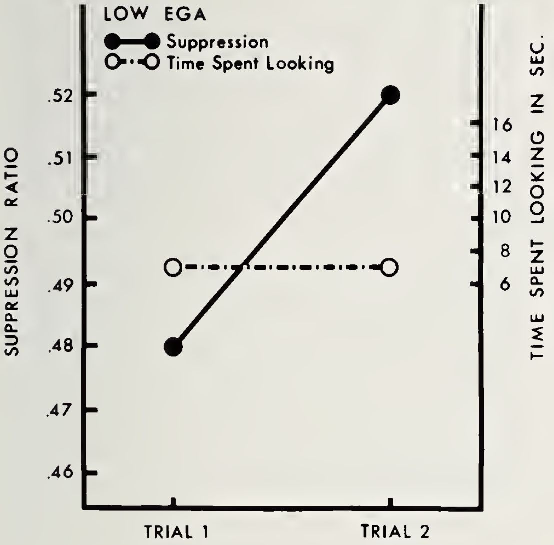

Figure 7–3. Suppression of non-nutritive sucking to and time spent looking at visual stimuli by infants born at a low estimated gestational age.

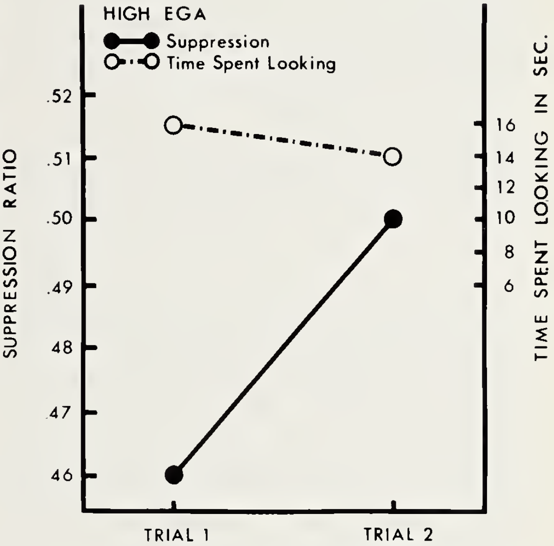

Figure 7–4. Suppression of non-nutritive sucking to and time spent looking at visual stimuli by infants born at term.

SUMMARY

The most important findings of the two studies can be summarized as follows: (1) The non-nutritive sucking pattern of human infants was different from that of the chimpanzee and the orangutan infants from birth. Human infants sucked in a rhythmical alternation of bursts and pauses, whereas the sucking of great ape infants can be better characterized as continuous sucking. These findings verify Wolff's report.[21] The pattern of non-nutritive sucking of human infants was stable from Day 1 on whereas the great ape infants did not show a stable pattern until approximately Day 5. The latter finding can probably not be generalized since this might be due to the conditions of the recording itself. The implications of this, however, will be discussed below. (2) The non-nutritive sucking patterns of LEGA and HEGA infants were very similar. This suggests that for human infants the pattern of non-nutritive sucking is well organized at a relatively early age and that it is, at least within the age limits studied, mature at birth, although some modifications, e.g. an increase in sucking rate, might occur within the first few days of life. (3) Great ape infants showed little or no suppression of non-nutritive sucking to auditory stimuli whereas human infants did show suppression. (4) Independent measures of visual behavior of human infants showed that the amount of sucking suppression seemed not related to the amount and quality of visual performance. Furthermore, within subjects, this suppression to visual stimuli recovered over trails while the visual behavior showed no such decrement in responding.

The finding that great ape infants suppressed their sucking little or none at all in response to the auditory stimulus is probably not due to an auditory deficit, since they are responsive to auditory stimuli at birth when not sucking. They show this responsiveness through hooting at the voice of the observer and reflexive twitching of the ears to tones. Furthermore, in the human subjects differences in visual behavior were also unrelated to the amount of sucking suppression.

There are probably a variety of other reasons for the findings summarized above. The non-nutritive sucking of the chimpanzee and orang-utan subjects showed a relatively long period of instability. This instability might have resulted from several factors. First, the obstetrical history of the great ape sample differed from that of the human sample. One chimpanzee was slightly premature, and two were born by caesarian section and were, as a result of drugs administered to the mother, severely depressed during the first few days of life. Kron, et al.,[14] demonstrated that newborn human sucking behavior is depressed for four days following delivery using routine maternal medication. Of the two orang-utan subjects, one was a month premature and had an immature sucking response both nutritively and non-nutritively (see Figure 7–5). The two full-term great ape infants (one orang-utan and one chimpanzee) did not exhibit this long period of instability, although they showed some. Second, the conditions of recording were not comparable between great ape and human subjects. A nipple designed for human newborns was used to record sucking of all subjects. Peiper[16] has suggested that the rubber nipple in comparison with

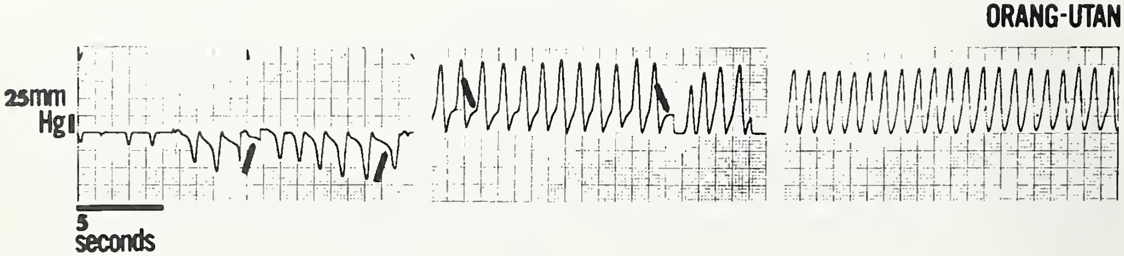

Figure 7–5. Analog records of the negative pressure component of non-nutritive sucking of a prematurely born orang-utan. The first recording was made at four hours, the second at two weeks, and the third at three months of age. (The negative pressure of the first recording was accidentally recorded as a downward deflection.) The negative sucking component was at first dysynchronous and executed in two phases. During the first phase the animal used its cheek muscles and during the second phase she dropped her lower jaw. Arrows indicate how the first phase is reflected in the analog record. These two phases have become synchronized at three months of age as illustrated in the third recording.

the breast nipple is a supra-normal elicitor of sucking for human infants. It has furthermore been found that the size and compressibility of the sucking instrument influences the rate of non-nutritive sucking in human infants.[6] This effect is even more pronounced with great ape infants whose sucking rate to oddly shaped nipples (e.g., a tube, or a nipple from which the elongated portion has been cut) is zero (unpublished data). The Davol nipple used in the recording of sucking therefore was probably not an optimal elicitor of sucking. Furthermore, sucking of all subjects was recorded while the infants were lying down. This seems to put the great ape infant at a particular disadvantage since their sucking is more stable when they are in an upright position and even hanging on to the experimenter, a more "natural" position for the great ape infant.

All of these findings point to the conclusion that the non-nutritive sucking response of the great ape infant seems to be a more rigid response than that of the human infant. It is more difficult to elicit, i.e., the conditions have to be just right, and once elicited it is more difficult to modify or to even stop the response to various stimuli. This statement is further supported by the results from recordings of non-nutritive and nutritive sucking of one chimpanzee over several months. These recordings showed that this animal did not begin to suck nutritively and non-nutritively in differential rates until one recording session at approximately three months of age. At the very next recording session and all subsequent sessions, the animal refused to suck non-nutritively. Figure 7–6 illustrates this phenomenon.

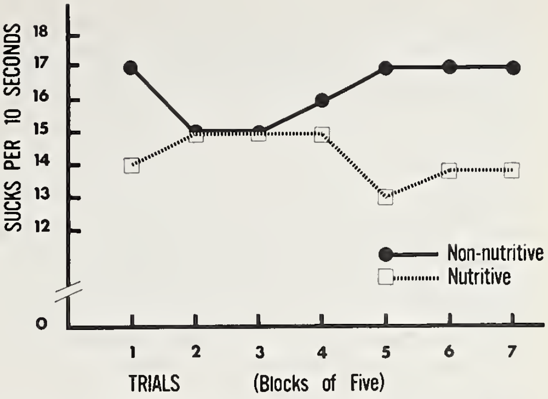

Figure 7–6. The first instance in which a three-month-old chimpanzee sucked nutritively and non-nutritively in differential rates.

As mentioned before, Wolff[21] suggested that differences in the sucking pattern between human and great ape infants represent a "qualitative change in central nervous system control over the sucking reflex." It was the intent of this paper to examine if non-nutritive sucking and the processing of external sensory information might not be influenced by the same underlying organismic factors. In other words, the question was asked whether the non-nutritive sucking pattern and visual behavior vary together.

The evidence from the second study, however, would indicate that the sucking response and the visual response vary independently. There seems to be a tacit assumption behind the use of a distraction paradigm with human infants that "the newborn is a 'one-track' enterprise, capable seemingly of one activity at a time."[4] (p. 7). Thus the infant can either suck or look but not suck and look simultaneously. The data on the human subjects presented in this paper, however, show that, within two trials at least, infants can "learn" to suck and look at the same time. Sucking suppression recovered over trials, while the amount and quality of visual behavior remained the same. This would suggest that the human newborn is at least a two-track enterprise. It, furthermore, opens up the possibility that great ape infants in contrast to human infants are not able to listen and suck simultaneously. The organization of the CNS could be such that the great ape newborn can only process one kind of input at any single time. The capacity to process information in more than one channel would give the human newborn a tremendous advantage over the great ape newborn.

More research with great ape infants is needed to examine this notion. Informal observations on a chimpanzee and a gorilla suggest that the visual behavior without sucking is about the same as that of the human infant whereas visual behavior while sucking is nonexistent. Furthermore, infants with suspected brain damage or mental retardation need to be looked at. It could be that the quality of the CNS is reflected in the number of channels the infant has available at birth for the simultaneous processing of information.

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13. Keen, R.: Effects of auditory stimuli on sucking behavior in the human neonate. *J. Exp. Child Psych.*, *1*:348–354, 1964.
14. Kron, R.E., Stein, M., and Goddard, K.E.: Newborn sucking behavior affected by obstetric sedation. *Pediatrics*, *37*:1012–1016, 1966.
15. Parmelee, A.H.: Sleep studies for the neurological assessment of the newborn. *Neuropädiatrie*, *1*:351–353, 1970.
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borns under visual and auditory stimulation. *Child Dev.*, *38*:443–452, 1967.

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DISCUSSION

STANLEY: Sucking in bursts and pauses during non-nutritive conditions by human infants is probably the major and most obvious difference between the sucking of human versus animal infants. We know, of course, that Wolff,[1] as Dr. Brown pointed out, has emphasized this difference.

However, I suspect that it may not be an all-or-none difference. Therefore, my question to you is: What do human infants do during their pauses in sucking that might be different from what animals do? Do they release the nipple completely? Do they relax?

J.V. BROWN: Even when they do not suck, human infants do not completely release the nipple during a pause. Some negative pressure is recorded during the pauses.

STANLEY: I have observed very young puppies suck in a short episode, drift off to sleep, then start sucking again, but we haven't studied this phenomenon systematically, as yet.

I suspect that this phenomenon may be related to the bursting and pausing of non-nutritive sucking in human infants. If so, some species differences in sucking, which now appear completely qualitative, may turn out to be more quantitative in character.

WEIFFENBACH: With reference to your first experiment, you seem to contrast the human infant's burst and pause pattern of non-nutritive sucking that is present from the earliest testing with the great ape infant's continuous pattern of non-nutritive sucking which is acquired during the early days of postnatal life. Does this imply that both classes of infants start life with a discontinuous pattern of non-nutritive sucking or are there reasons to believe that the pattern of continuous non-nutritive sucking of the great ape is, in fact, available congenitally?

J.V.BROWN: I believe that the latter is probably true. As I mentioned in my talk, there are a variety of factors which can account for the initial instability of the sucking response of my great ape newborns.

WEIFFENBACH: A second question has to do with the ontogeny of the relation between patterns of non-nutritive and nutritive sucking. Using a simple description like "continuous" versus

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[1] Wolff, P.H.: The interaction of state and non-nutritive sucking. pp. 293–310, in Bosma, J.F. (Ed.): *Third Symposium on Oral Sensation and Perception: The Mouth of the Infant.* Springfield, Thomas, 1972.

"discontinuous", one could assert that there is concordance between nutritive and non-nutritive sucking patterns for the human infants from the beginning. After the immediately postnatal period the great ape infants show similar concordance of pattern. What about the relation of nutritive to non-nutritive sucking in the first few days of life, before the continuous pattern is shown in non-nutritive sucking?

J.V. BROWN: When great ape infants show unstable sucking which is recorded as "discontinuous" sucking, they do so both nutritively and non-nutritively. When they shift to a continuous, more stable pattern, they again do this nutritively and non-nutritively. This development is different, I believe, from that of the human newborns. The latter suck non-nutritively in a discontinuous pattern from birth while nutritive sucking becomes more continuous as the infant becomes stronger.

SAMEROFF: Depending on the volume of the nipple, expression, that is, squeezing, will produce different recorded pressures. If different nipples are used to feed different animals, resulting differences in pressure may be a function of the nipple. Using the same volume nipple across species controls for this possible artifact.

J.V. BROWN: The same physical nipple, however, might not be comparable across species. As I said before, I do not believe that a Davol nipple is an optimal nipple for chimpanzee and orang-utan infants.

BOSMA: Note that Dr. Sameroff is referring to positive pressure variations in sucking, but Dr. Brown is recording only negative pressures.

J.V. BROWN: I only recorded negative pressures.

MISTRETTA: Have you tried your experimental auditory stimulus on a newborn ape which is not sucking, to determine if the unoccupied infant will attend to the stimulus?

J.V. BROWN: I have only informal observations. The newborn ape will turn his head in the direction of a sound stimulus while he is not sucking and he will hoot at you when you talk or hoot at him. But I need to make more formal observations.

CAMPBELL: One thing occurred to me while you were talking: would it be a fair description to say that the apes that you were looking at have only the nutritive sucking form that you see in the human infant. That is to say, long bursts of sucking without switching off, which is the human nutritive sucking pattern.

What I was thinking was this, you said that maybe the apes only have one channel of input. I think in terms of experiments on skilled behavior, where you ask the human operator to take information in from a variety of sources, the thing that he does is to look at one thing at a time, and then switches. What controls the behavior is switching.[1] I was wondering if perhaps the human infant isn't perhaps better at switching; finds something in his mouth, sucks to investigate it, discovers it is nothing, and then switches off, starts again, and so on. You get the non-nutritive sucking pattern, on-off, on-off.

When it delivers something, he is monitoring his performance as satisfactory, because something is arriving at the mouth, and then you get the long bursts.

The ape, perhaps, is hung up with the stimulus in his mouth, starts to suck, and can't stop.

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[1] Broadbent, D.E.: *Perception and Communication.* Oxford, Pergamon, 1958.

J.V. BROWN: I think you might be right. The sucking behavior of the great ape infant is different than that of the human infant. On the one hand the ape needs more priming in order to get his behavior going, on the other hand, once he is sucking he has a harder time to stop. Without independent measures, however, it is difficult to say if the great ape does not attend to auditory stimulation while he is sucking or if he does attend but is simply incapable of switching the sucking response off.

CAMPBELL: What is the nature of the ape's — the infant ape's attentional behavior, let's say, when he is looking, when there is something for him to look at but nothing for him to suck?

J.V. BROWN: I have found good visual fixation and tracking in the infant ape when he is not sucking.

CAMPBELL: It is difficult then to get him to listen to something?

J.V. BROWN: It is easy to get him to listen to auditory stimuli while he is not sucking. However, I should compare the auditory and visual behavior of the great ape with and without non-nutritive sucking in order to get some more information about the great ape's attentional behavior.

BOSMA: At what age does the ape begin to twitch his ears in response to sound?

J.V. BROWN: Right after birth.

BOSMA: Does the infant ape twitch his ears in response to sound but not interrupt his suckle?

J.V. BROWN: He does not twitch his ears while he is sucking.

BOSMA: Dr. Campbell, does this observation contribute to your point about the dominance of oral experience during sucking in the infant ape?

CAMPBELL: Yes, this is the sort of thing I had in mind.

STARK: In the first experimental situation, do you think it would be meaningful to compare the responses of the infants of each species to the sounds typically produced by the mother, that is, to the sounds peculiar to mother-infant communication for each species.

BOSMA: May I add another parameter of consideration as you observe the effects of auditory stimulation on infant mammals. The auditory competence of the infants of different species may be notably different. In working with the young of different mammals, it is important to know their competence in upper frequency ranges. There may be communications which are entirely impertinent to us and critical to them.

J.V. BROWN: In response to Dr. Stark, I do not know if it is meaningful to present identical auditory stimuli to subjects of different species. There is certainly some data[1] that would suggest that human newborns are particularly responsive to auditory stimuli which approximate the female voice. By analogy, one would expect great ape infants to be more sensitive to auditory stimuli close to those emitted by members of their own species. This points up one of the problems facing investigators who are interested in comparative research. Does one keep the physical stimulus the same, hoping that the responses to this stimulus will be more or less comparable, or does one present a different stimulus to subjects of each species, hoping that each will be maximally effective? Either way one probably loses some. As far as Dr. Bosma's remark is concerned, I do not believe much is known about the auditory competence of newborn great apes.

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[1] Hutt, S.J., Hutt, C., Lenard, H.G., von Bernuth, H., and Muntjewerff, W.F. Auditory responsivity in the human neonate. *Nature: 218,* 88–890, 1968.

STARK: Also it has been shown in human infants of only 4 weeks that the rate of non-nutritive sucking changes in response to presentation of synthesized speech sounds,[2] and also to the presentation of the same sounds produced by real speakers.[3]

STEINER: Regarding your methods, I would remark that the visual stimuli you used are patterned, while your acoustic stimulation is unpatterned. Perhaps by using intermittent constant illumination and darkness periods you would create more comparable experimental conditions.

J.V. BROWN: I did not make comparisons between auditory and visual suppression because they cannot be compared in this experimental system.

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[2] Eimas, P.D., Siqueland, E.R., Jusczyk, P., and Vigorito, J. Speech perception in infants, *Science, 171,* 303–306, 1971.

[3] Trehub, S.E., and Rabinovitch, M.S., Auditory-linguistic sensitivity in early infancy. *Develop. Psychol.* 6, 74–77, 1972.

Chapter 8

REFLEXIVE AND OPERANT ASPECTS OF SUCKING BEHAVIOR IN EARLY INFANCY

ARNOLD J. SAMEROFF

Research on the infant during the first months of life derives both its interest and fascination from the observation of the transition of an organism from a biological mode of functioning to a psychological one. The interface of these two modes seen in the newborn period requires the researcher to be sophisticated both in the physiological roots of sensory and motor behavior in the fetal period and the psychological consequences of their coordination and integration during infancy.

Past attempts to explore the psychological world of the young infant were made difficult by the apparent paucity of behaviors on which to focus. I say "apparent" rather than "real" because the deficit seems to have resided more in the technological inadequacies of the researcher rather than in the response inadequacies of the subject. During the last decade a rapid upsurge in research on early behavior has resulted from the development and use of sophisticated electronic instrumentation which has allowed investigators to assess a wide array of electrophysiological, autonomic, and motoric behaviors.

Among these behaviors, sucking has received a great deal of attention because of its centrality to infant functioning. Physiologically, sucking behavior involves complicated integrations of mouth, cheek, respiratory and swallow responses. Psychologically, sucking behavior appears to be experientially modifiable as a function of its connection with other behaviors such as head turning and search behavior, and its ultimate supercession by other modes of eating behaviors.

The bulk of research on sucking behavior can be broken into three categories: (1) Research focused on the description of the response and how its components are coordinated with each other and with other response systems involved in the sucking process, e.g., swallowing, breathing, and sleeping, (2) Research on the plasticity of the sucking response, studying its adaptability under varying environmental contingencies as in conditioning studies, and (3) Research using sucking as an index of other psychological functions, e.g., attentional processes. The following sections will deal with each of these three aspects. But before engaging the data of sucking research a prefatory word is necessary about one's conception of the infant in relation to this environment.

ADAPTATION AND ACTIVITY

Two points will be made in the following discussion which emphasize the continuity of developmental functioning that characterizes the infant's passage from zygote to fetus to child. The work of the embryologists presented at this symposium[13] provide an appropriate model for the psychological considerations raised here.

In regard to adaptation, the biological development of the child cannot be seen as determined by either his genetic structure nor by his environmental circumstance. Induction and transplant studies in embryology[25] have shown the intimate relationship between organism and environment in which a change in either can modify the other. Both the organism and his environment are then seen to be in a constant state of adaptation to each other. These same principles of biological functioning have been extended by such theorists as Piaget[15] and Sameroff and Chandler[23] into the realm of psychological functioning.

In contrasting a realm of biological functioning with one of psychological functioning it would be appropriate to define these disciplines. Although psychology has been generally defined as the study of behavior, this definition must be delimited for our use. A more restricted definition would be the study of changes in behavior which are the result of experience. But again we are faced with defining experience in a way which would make the distinction meaningful in separating psychological from biological functioning. At the same time one must be careful not to define psychology as independent of biology since without the organic basis of physiological functioning there could be no psychology.

A definition which maintains the continuity of functions from biology to psychology but at the same time emphasizes their contrast would require a distinction between the material adaptation between the organism and his environment found in biological functioning and the informational adaptation found in psychological functioning. Psychology is then seen to be concerned with the organization of information. Initially, information is directly extracted from the organism's external environment, but at more advanced levels of functioning, psychological behavior is almost completely intrapsychic when such processes as hypothetical or mathematical thinking occur.

The fascination of the period of early infancy is that it is at this point in time that the transition occurs from the primarily biological *material* development of fetal life to the beginnings of the psychological *informational* functioning which at its highest levels of organization will characterize the uniqueness of human behavior. The research to be reported here takes as its focus the emergence of the organization of behavior based on informational rather than material transactions between the organism and its environment.

The two points of continuity to be emphasized are, first, that in both biological and psychological functioning adaptation is the functional process by which the organism integrates itself with its environment and, secondly, as a corollary, the adaptation process is a function of an "active" organism.[11] It is the organism's activity which provides the motive force behind the change in both material and informational organization.

BIOLOGICAL BASIS OF SUCKING BEHAVIOR

Before dealing with the adaptation of the sucking response, the physiological functioning of the various components and types of sucking will be described. The human sucking response appears to be organized differently from that of other animals, even other primates. Wolff[26] and Brown[3] have reported that the human is the only species to have a different temporal pattern for nonnutritive as contrasted with human nutritive sucking or with both nutritive and non-nutritive sucking in other animals. Where in human nutritive sucking there is initially a continuous period of sucking which lasts for a number of minutes, in nonnutritive sucking the pattern is an alteration between a burst of some 5–20 sucks, lasting around seven seconds, followed by a no-sucking interval of equal duration.

There has been speculation that the manner in which the human infant gets milk from the nipple may be at variance with techniques used by other animals. Ardran, Kemp, and Lind,[1] using cineradiographic techniques, showed that in human nutritive sucking the tongue was used to strip milk from the nipple by applying pressure to the underside of the nipple and moving the tongue backwards. This "expression" component of the sucking response appears quite similar to sucking found in other mammals.

However, Colley and Creamer[4] analyzed the pressure in the infant's mouth during sucking and found a strong negative suction as a consequence of the infant lowering the floor of his mouth. These authors maintained that the negative pressure of this "suc-

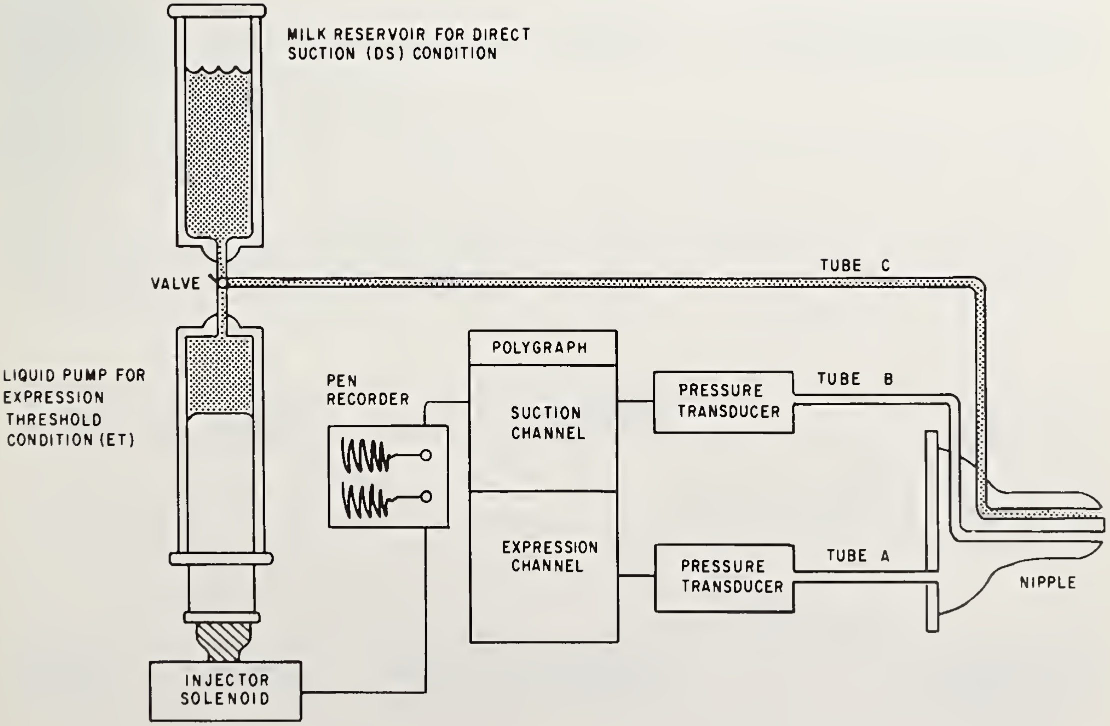

Figure 8–1. Experimental nipple and milk delivery system.

tion" component of the nutritive sucking response had a greater role in getting milk then any action of the tongue.

Sameroff[17] attempted to resolve the issue by simultaneously measuring the pressure exerted by the tongue on the nipple and the negative suction pressure in the mouth, i.e., the expression and suction components, respectively. A special nipple was designed based on earlier work by Kron, Stein and Goddard[12] (See Figure 8–1). The nipple had three tubes connected to it. One tube measured the pressure inside the nipple which would be analogous to the squeezing pressure of the expression component. A second tube passed through the nipple and a short distance out through the tip in order to measure the suction pressure applied to the nipple. A third tube which also passed through the nipple and out to the tip was used to deliver milk to the infant. The milk delivery system was arranged so that it could be a consequence of either component of the sucking response, expression or suction.

When the apparatus is placed in the Direct Suction mode, the infant obtains milk from the reservoir through Tube C in Figure 8–1 by generating negative suction pressure in the mouth. In this mode the infant's ability to maximize his suction pressure can be observed as well as whether he continues to use the irrelevant expression component of sucking, that is, squeezing the nipple.

When the apparatus is placed in the Expression Threshold mode, the pressure amplitude of the expression component of sucking is measured via a pressure transducer. When the pressure exceeds a threshold condition (usually 25 mm of Hg), the liquid pump injects 0.1 cc of milk into the infant's mouth, the amount typically obtained from one suck in the Direct Suction mode.

Similarly, when the apparatus is placed in the Suction Threshold mode, the pressure amplitude of the suction component of the sucking response is measured and when it exceeds a specified threshold, milk is injected into the infant's mouth. The contrast between the Direct Suction and Suction Threshold modes was that in the former the amount of milk received was directly proportional to the amount of pressure, in the latter the amount of milk was the same as long as the pressure threshold was exceeded.

Figure 8–2 is a record of the expres-

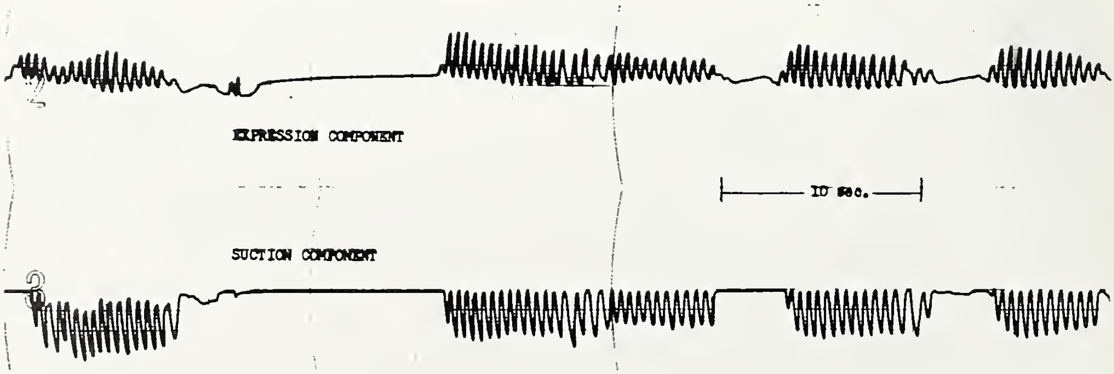

Figure 8–2. Non-nutritive sucking record for a single subject.

sion and suction components when no milk is being delivered. The burst-pause pattern of the non-nutritive sucking, which Wolff[26] has described as uniquely human, can be clearly seen. Although human nonnutritive sucking can be differentiated from other mammals by the burst-pause pattern, it is less clear that it can be distinguished from human nutritive sucking in the same way.

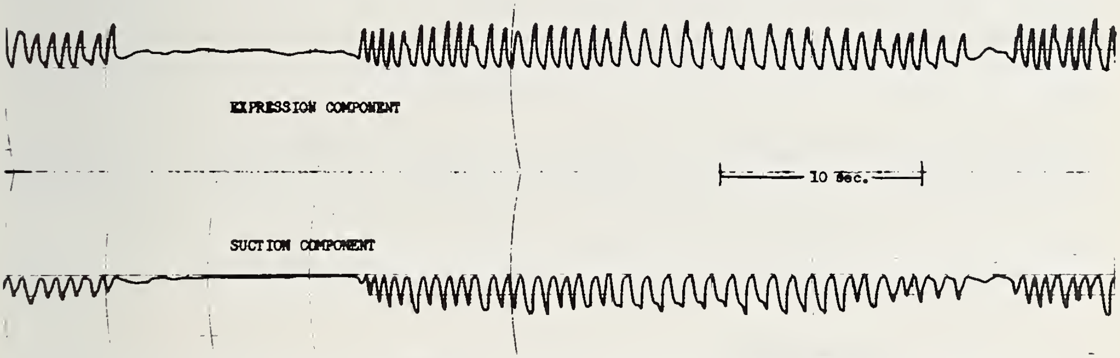

Figure 8–3. Nutritive sucking record for a single subject in the Direct Suction condition.

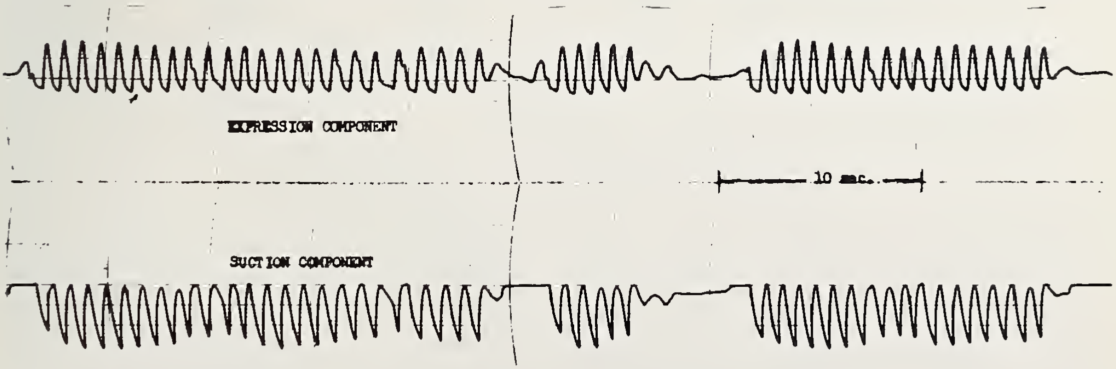

Figure 8–4. Nutritive sucking record for a single subject in the Expression Threshold condition.

Figures 8.3 and 8.4 show human nutritive sucking in the Direct Suction and Expression Threshold mode, respectively. Although the bursts of sucking are longer than in the nonnutritive condition (the first burst may run as long as three minutes), the burst pause pattern is still evident. One could argue that both nonnutritive and nutritive sucking in humans are different from that of other mammals.

Other differences between nutritive and nonnutritive sucking can be seen in the slower rate of sucking and higher rate of swallowing in the former. Swallows can be seen in the figures as bumps on the downside of the sucks. The explanation for the difference in sucking rates between nutritive and nonnutritive sucking is not clear. It may be related to the necessity for coordination with respiration and swallowing in the feeding process.

When obtaining milk is made contingent on the suction component as in Figure 8.3, the amplitude of the suction component can be seen to be higher than in Figure 8.4, where milk delivery is contingent on the expression component.

The suction component seemed to be tied to the expression component of sucking. Suction rarely occurred without expression. Only two of 25,000 suction responses were found to be unaccompanied by an expression component. On the other hand the expression component is not so closely tied to suction. In the Expression Threshold condition the expression component frequently occurred in the absence of the suction component (see Figure 8.5).

To summarize, human nutritive sucking appears to be differentiated from nonnutritive sucking by a slower rate, longer bursts, and more swallowing. Within nutritive sucking there seems a necessity for the suction component to be accompanied by an expression component. The reverse seems to be less the case.

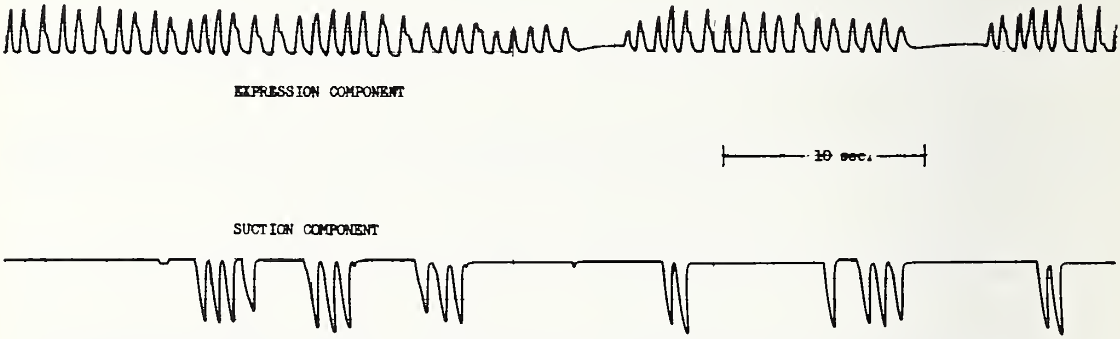

Figure 8–5. Nutritive sucking record for a single subject in the Expression Threshold condition (note absence of suction component).

PLASTICITY OF THE SUCKING RESPONSE

The second issue to be addressed is the determination of the plasticity of the sucking response. If the child has different contingencies associated with different parameters of his sucking behavior, will he be able to modify his performance as a function of these contingencies? A series of studies in our laboratory explored these questions.[17,21]

In each of the three studies to be described bottle-fed newborns were seen at their 2:00 AM and 6:00 AM nighttime feeding periods when they were between two and five-days old. These babies had their first feeding 12 hours after birth, so that by the time they were seen in the experimental situation they had had between 10 and 30 prior sucking experiences. Halverson[7] had found that while some prematures showed discoordination in their nutritive sucking behavior, after the first few feedings, most full-term infants were well organized in this respect. In the studies reported here, a population was used that had already coordinated their sucking behavior and adapted to bottle feeding. Since these infants had had prior nutritive experience, one must be careful in the interpretations one can make about differences found in sucking behavior during the test situations.

Plasticity of behavior could result from prior adaptability built into the sucking response. The infant when placed in the new situation would be seen to immediately modify his performance to optimize milk-getting. One would not know whether to attribute this adaptability to some innate capacity or to the previous experience of the newborn. To demonstrate that the newborn could alter his sucking in the face of new contingencies, changes would have to occur during the experimental session. In other words sucking behavior at the end of the experiment should be different from that at the beginning. An even stronger case for the infant's adaptive processes could be made if modifications that occurred during the first experimental feeding period were maintained across the 4-hour interfeeding interval and were to be evident at the beginning of the second feeding period. Unfortunately the carry-over of changes in performance did not occur.

Three studies were completed which included a variety of contingencies to evaluate the infant's ability to modify his behavior. In each session, the first minute of sucking was non-nutritive, i.e., no milk was delivered through the nutrient delivery tube. During the next 5-minute period, the infant was given milk under the appropriate response condition. Direct Suction or Expression Threshold in Study 1, high and low Expression Threshold in Study 2, and high and low Suction Threshold in Study 3. In Study 1 this 6-minute cycle was repeated, while in Studies 2 and 3, after the first non-nu-

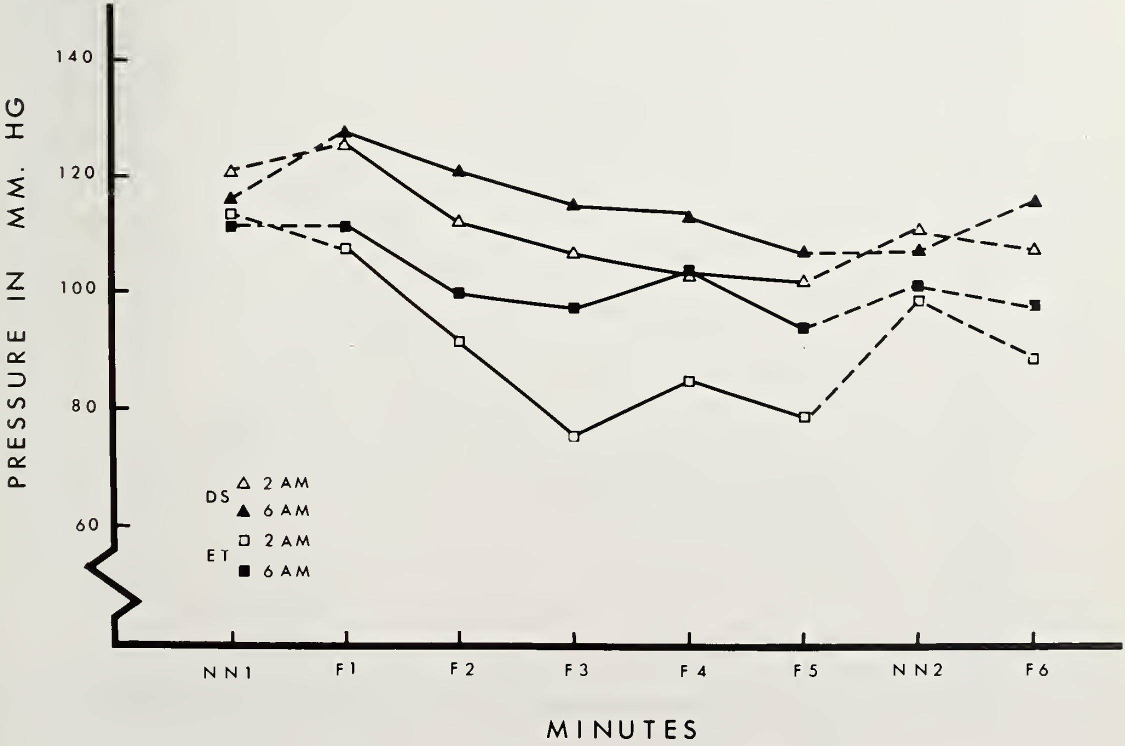

Figure 8–6. The suction amplitude of sucking responses during an observation of feeding for infants in the expression threshold condition (ET) and the direct suction condition (DS). A minute of non-nutritive sucking (NN1) was followed by five minutes of feeding (F1 - F5), another minute of non-nutritive sucking (NN2) and another minute of feeding (F6).

tritive minute the infant was fed throughout the session.

In the first study a comparison was made between infants fed in the Direct Suction condition and infants fed in the Expression Threshold condition at two successive feedings. The infants gave evidence of adaptability to the contingencies of the feeding situation. They sucked differently in the two conditions.

In the Direct Suction condition where milk was delivered contingent on the performance of the suction component, the infants had higher amplitudes of suction than in the Expression Threshold condition. These differences appeared in the first minute and were interpreted as the result of prior adaptations of sucking (see Figure 8.6).

In the Expression Threshold condition, changes in the suction component occurred which appeared to be a consequence of the new experience in the feeding (see Figure 8.7). While in the first minute there was no differences in the number of suction responses between the two conditions, during the five-minute feeding period fewer and fewer suction responses occurred so that by the fifth minute only 80% of the ex-

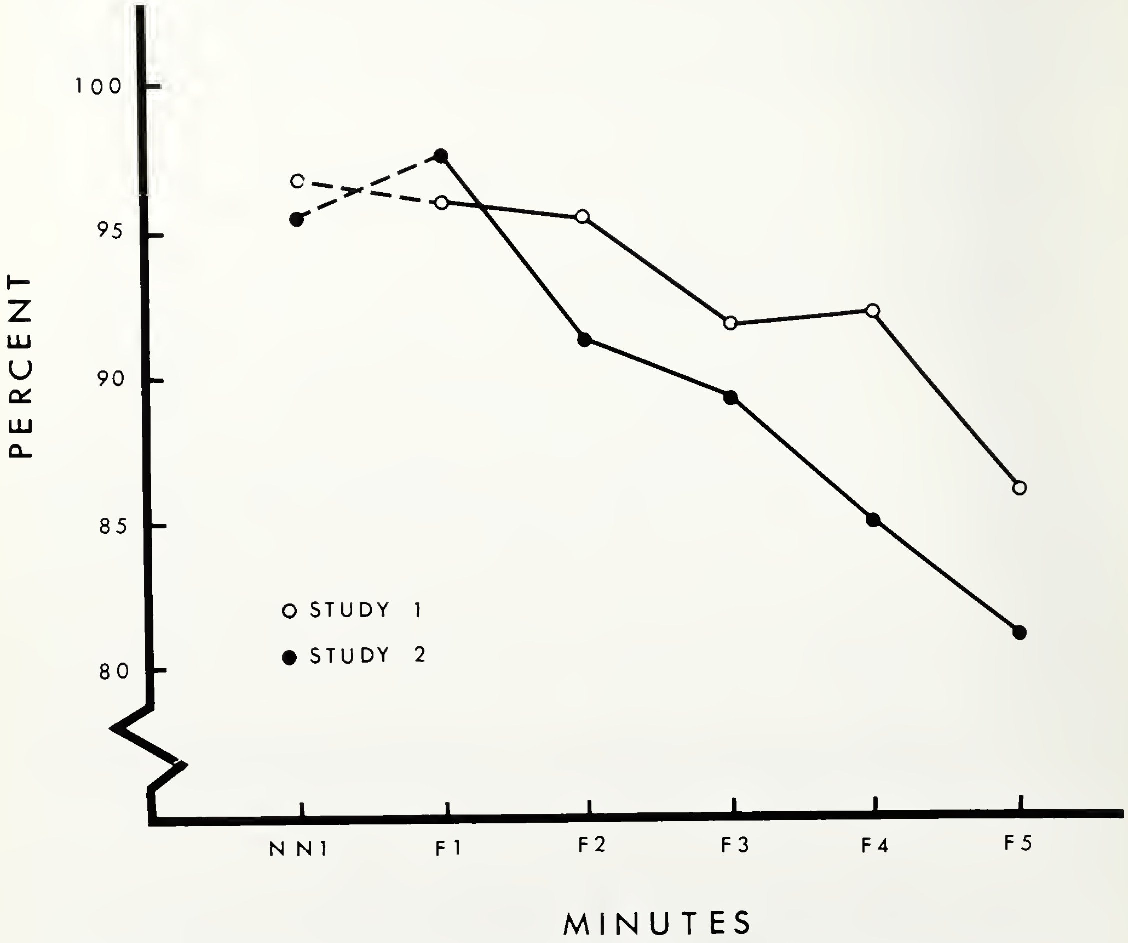

Figure 8–7. The ratio of the suction component to the expression component over time in the expression threshold condition (ET) for Study I and Study II.

pression responses were accompanied by a suction component. This change in ratio seemed to be evidence for new adaptations of the sucking response to the novel contingencies in the experimental situation.

The amplitude of the expression component did not change in the expected direction to the feeding contingencies. The expression amplitude was less in the Expression Threshold condition where milk was contingent on the expression component (see Figure 8.8). A potential explanation for these unexpected results was that the infant had optimized his performance to match the required threshold for milk delivery (25 mm of Hg). Expressing with a greater amplitude would produce no greater quantity of milk.

Study 2 was designed to test this hypothesis. Two different expression amplitude thresholds were used. Half of the infants were fed in a low threshold condition (25 mm of Hg) at their first feeding and in a high threshold condition (50 mm of Hg) at the second. For the other half of the infants, the order was reversed.

When the expression amplitude was compared in these two conditions, it was found that the infants responded differently in the two situations and, moreover, the differences seemed to increase across the feeding session giving further evidence of new adaptations of the sucking response (see Figure 8.9).

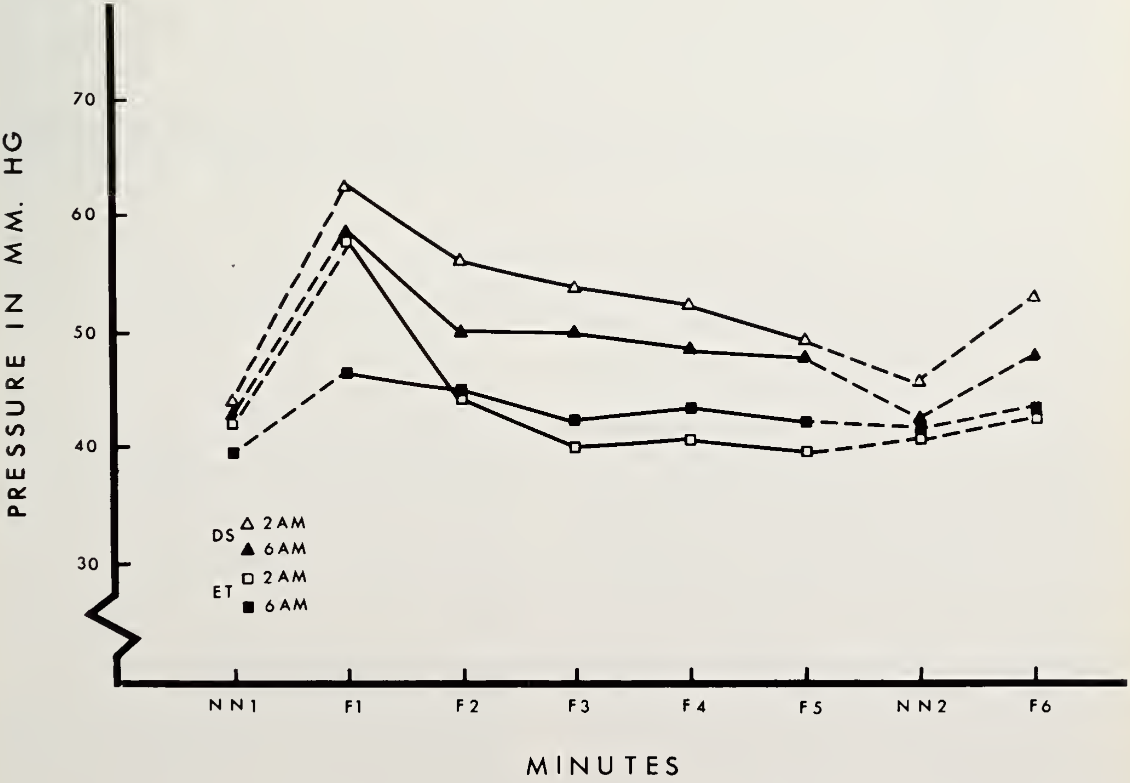

Figure 8–8. The expression amplitude of sucking responses during an observation of feeding for infants in the expression threshold condition (ET) and the direct suction condition (DS).

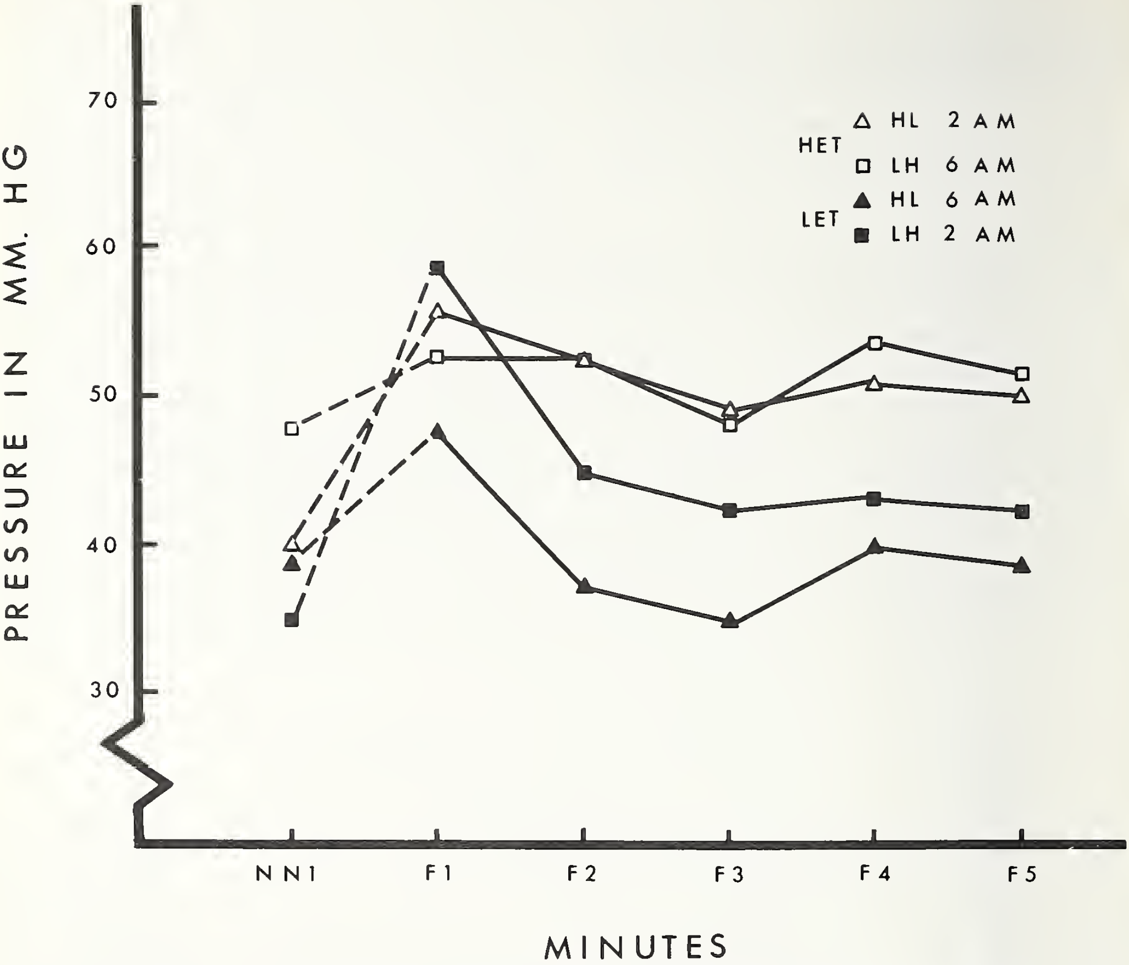

Figure 8–9. The expression amplitude of sucking responses during an observation of feeding for infants in the high expression threshold condition (HET) and the low expression threshold condition(LET).

In Study 3 an attempt was made to manipulate the suction amplitude in a similar fashion. Infants were fed in a Suction Threshold condition in which the required Amplitude Threshold was 100 mm of Hg at one feeding and 50 mm of Hg at the other (see Figure 8.10). Although the differences in suction amplitude were in the expected direction they were not large enough to be statistically reliable. A selection of higher thresholds might have produced more differentiated performance in the two Suction-Threshold feeding conditions.

The results of these investigations demonstrated that the newborn human infant can change his sucking behavior as a function of the consequences of his activity. Study 1 showed that the components of the sucking response can be influenced by varying the relation of the components of sucking to the delivery of nutrient. If nutrient delivery is directly contingent on the performance of the negative pressure component of sucking, the newborn uses more negative pressure than when nutrient delivery is not contingent on this component. If

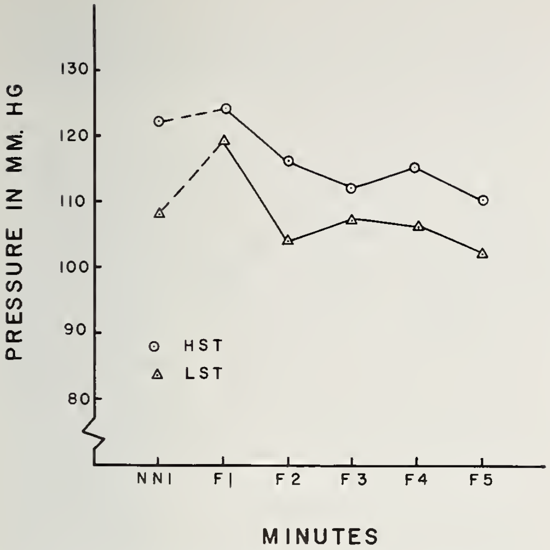

Figure 8–10. The suction amplitude of sucking responses during feeding in a high suction threshold condition (HST) and a low suction threshold condition (LST).

nutrient delivery is contingent on the expression component, the newborn performs the now unnecessary negative pressure component less often. Study 2 was designed to investigate further the positive pressure component of sucking. Again it was demonstrated that the infant exhibited a relatively high amplitude of expression pressure. When a strong pressure is required for the delivery of nutrient, the infant exhibits a relatively high amplitude of expression. When a lower amplitude is sufficient to obtain nutrient, the infant exhibits a lower amplitude of the expression component.

Most of the effects found in the two studies seem to be the result of previously organized abilities of the newborn to adapt his sucking response. The differential training in the first session had no measurable influence on the performance of the sucking response in the second session. In Study 1 when differences between the groups occurred, the differences appeared within the first minute, remained constant until the nonnutritive period, in which they disappeared, and then reappeared during the first minute of renewed feeding.

There were, however, two changes in performance that may have been the result of new adaptations of the sucking response. In both studies, the linear function shown by the increasing inhibition of the negative pressure component during the expression threshold conditions and in Study 2 the change of positive pressure amplitude in the low expression threshold condition were different from the immediate changes found in the amplitude measures of Study 1. The fact that the response modifications in the various studies did not persist from the first session to the second indicates that the new behaviors had not had the opportunity to stabilize adequately. Although longer training was not possible at the time of the present study because infants were available only during their two nighttime feedings, longer experience in the different conditions would seem an appropriate direction for future research.

ORIENTATION AND THE SUCKING RESPONSE

In the previous two section, the sucking response has been discussed in terms of its topology and its sensitivity to alterations in feeding contingencies. The sensory-motor coordinations necessary in order for the infant to adapt his responding are closely connected in that the oral motor behavior is closely tied to the oral sensory behavior. Changes in sucking activity were related to changes in the exteroceptive cues from milk in the mouth along with the additional proprioceptic cues of accompanying swallowing and respiratory behavior.

Attempts by other investigators to relate sucking behavior to inputs from more distant sensory organs, e.g., auditory and visual, have been less successful. Sameroff[21] reviewed much of these studies and concluded that there was little evidence that human infants were able to alter sucking behavior in classical conditioning research paradigms. These studies had required integration of the sucking response with auditory or visual stimulation.

A different approach that has been taken to the relation of sucking to sensory input has been to place it in the context of a general orienting reaction.[2] Sokolov had proposed a configuration of autonomic, electrophysiological, and motor responses which he called the orienting reflex.[24] Bronshtein[2] utilized sucking as an index of the orienting reaction. Cessation of sucking behavior in response to exteroceptive stimulation was thought to be a component of a more general cessation of motor activity which occurred when animals oriented. Although Bronschtein and her associates claimed to have demonstrated sucking cessation during auditory stimulation, attempts to replicate these results have been generally unsuccessful.[8,10] The only study which reported reliable sucking cessation, used moving visual stimuli.[6] The contrast in results as a function of modality of stimulation will be discussed later in the paper. Using newborn infants, some exploratory research in my laboratory failed to find the cessation effect even when very strong stimuli were applied, e.g., 100 decibel tones. Because of these failures to find immediate effects of stimulation on the sucking response, a new study was designed in which the focus was shifted to longer duration effects of stimulation on the sucking response.[16]

Non-nutritive sucking was monitored in newborn infant while a series of 17 60-second auditory and visual stimuli were successively presented. When the seconds of sucking were calculated for each 60-second period, no differences were found between the various stimulus conditions. A second analysis evaluated the pattern of the sucking ignoring the total amount of sucking. During each 60-second period the number of sucking bursts were counted. Figure 8–2 shows the burst-pause pattern typically seen in non-nutritive sucking. Figure 8.11 shows the changes in number of bursts per minute when the infant was experiencing different stimulus conditions.

When compared to a control condition with a moderate illumination level, presentations of either tones, bright lights, or total darkness decreased the number of sucking bursts. It appeared then, that at least one parameter of the sucking response was sensitive to the effects of distant exteroceptive stimulation. While the quantity of sucking, i.e., seconds of sucking, did not differentiate between stimulus conditions, the quality of sucking, i.e., the burst-pause pattern, did.

With this new insight into the organization of non-nutritive sucking, another study was designed to seek the Bronschtein sucking cessation effect in newborns.[18] Rather than a time-dependent stimulus presentation, e.g., every 20 seconds independent of what the infant was doing, the stimulus presentation was made to be response dependent. Stimulus onset occurred either at the beginning of a sucking burst or at

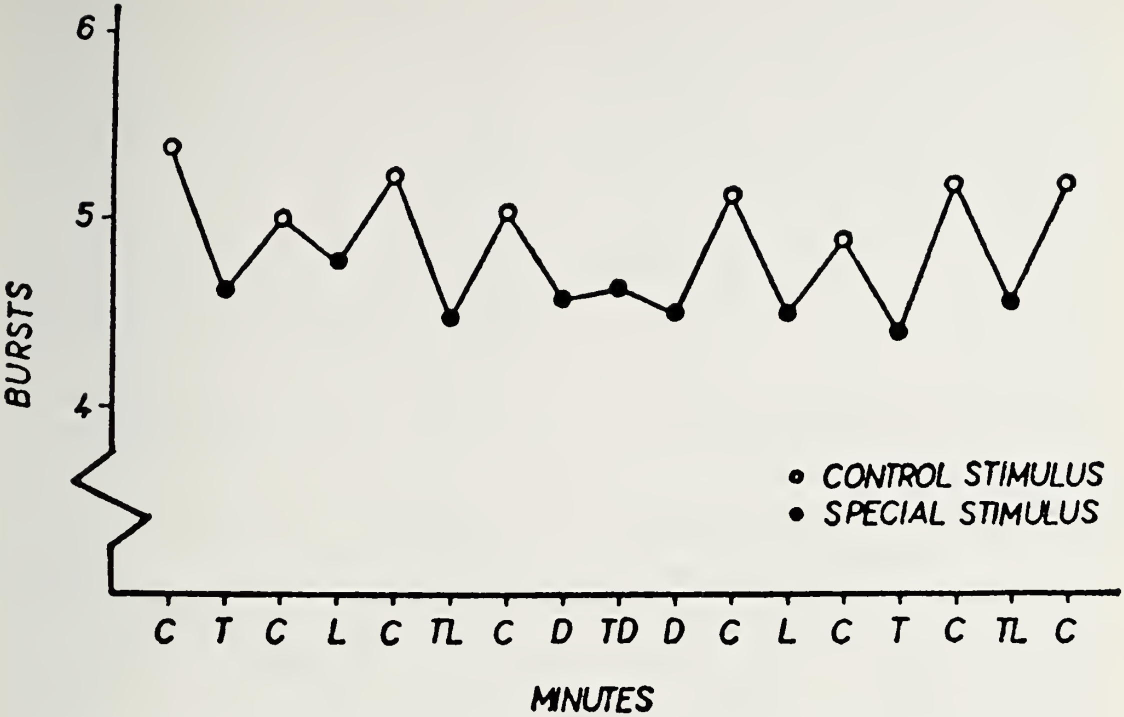

Figure 8–11. The mean number of sucking bursts per minute during stimulation in a control condition (C), tone (T), bright light (L), tone and light combined (TL), darkness (D), and tone and darkness combined (TD).

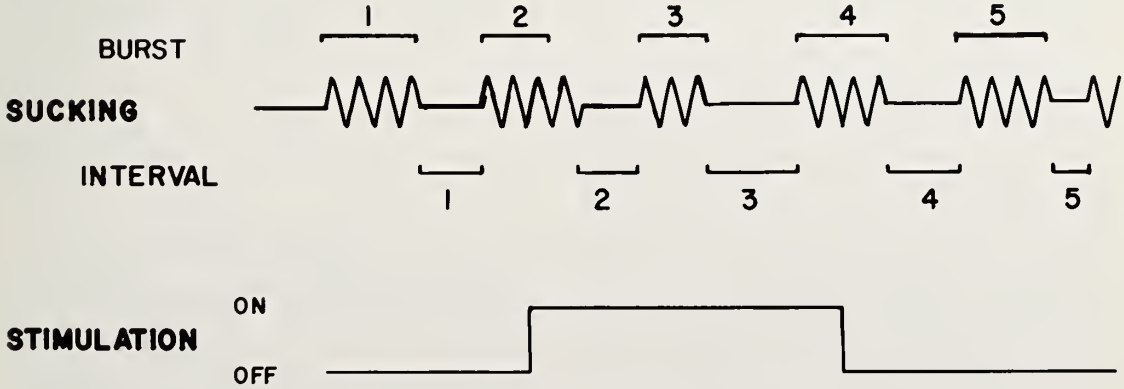

Figure 8–12. Paradigm and units of analysis for each stimulus trial.

the beginning of an interburst interval. The experimental design can be seen in Figure 8.12. Each trial consisted of five bursts. Stimulus onset occurred during the second burst and offset during the fourth burst. To test for the effects of stimulus onset or offset, comparisons could then be made with the preceding or following burst during which no stimulus changes occurred. Each infant received four presentations of each of four 500 Hz auditory stimuli: a constant 65 db tone, a constant 75 db tone, a 65 db tone which was alternately on and off at

250 msec intervals, and a 65 db tone that was on and off at 500 msec intervals. The infants were seen during sessions on four consecutive days. The effects of stimulation on the duration of sucking bursts can be seen in Figure 8.13.

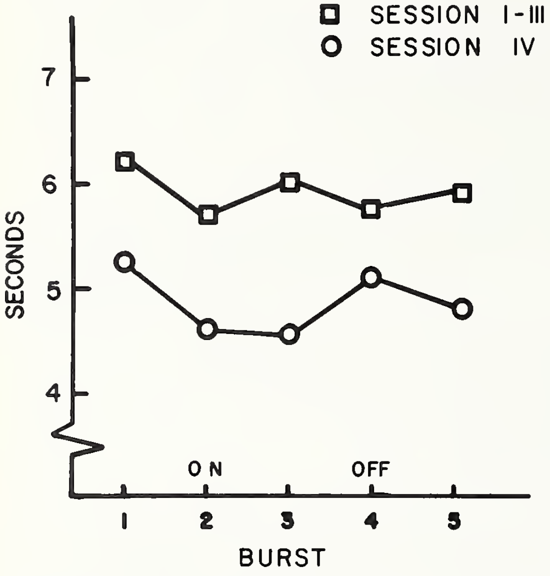

Figure 8–13. Mean sucking responses to onset and offset of stimulation.

The data from the trials in each of the sessions were averaged together, since no differential stimulus effects had been found. A statistically reliable reduction in burst duration was found. The burst in which stimulus onset occurred was shorter in duration then than the preceding burst in which no stimulus change occurred. However, this effect was not the dramatic cessation described by Bronschtein and her associates.

Following this failure to clearly replicate Bronschtein's sucking cessation effect in newborns, I reread the Bronschtein et al.[2] article and found that not only newborns but older infants had been used in the study. The older infants were reported as showing much larger cessation of sucking effects than the newborns. As a consequence another study was designed in which older infants were used.[19]

Infants at one, two, and three months of age were presented with the same series of auditory stimulation used in the preceding study.[18] Trials were again five bursts in duration with stimulus onset occurring during the second burst and stimulus offset during the fourth burst (see Figure 8.14). As in the previous newborn study infants at one and two months of age did not show a large sucking cessation effect, i.e., there was no reliable difference between the duration of the sucking burst in which stimulus change occurred and the preceding sucking burst during which no stimulus change occurred.

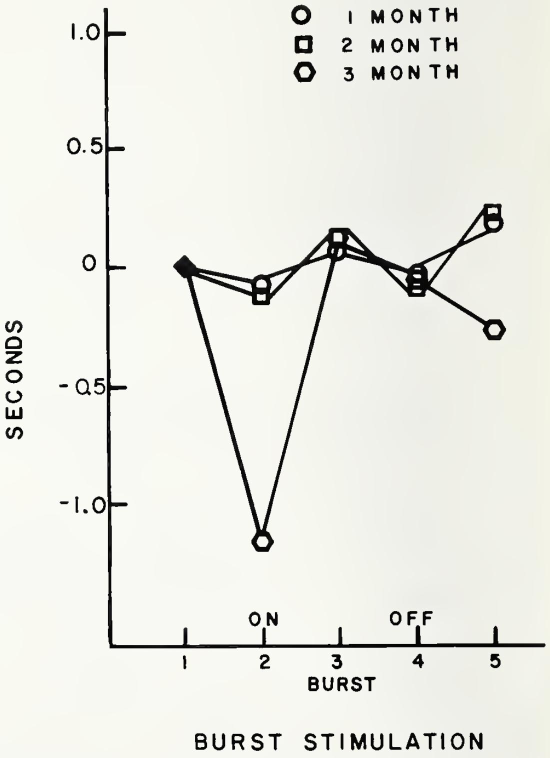

Figure 8–14. Average sucking burst response to stimulation for Ss at each age level.

However, in the group of three-month old infants large sucking cessation effects did occur to stimulus onset. It appeared, then, that between the second and third month of life a change occurred in which sucking cessation became a reliable indicator of stimulus change.

The source of the change in effect of stimulation with age can be attributed to a variety of sources. The most readily available explanation would be a lack of maturation in the physiological structures necessary for attentional mechanisms. However, it has been demonstrated that under appropriate conditions newborn human infants would show orientation responses.[9,22] An hypothesis which seems to be gaining in credibility is that the state of the infant plays a major role in his behavioral capacities.[5] The newborn is generally asleep, drowsy, or crying, and is only rarely awake and alert. Studies which have not used awake, alert infants have generally not found orientation reactions.[5] Studies which have required newborns to be awake and alert have been more successful.[9,22]

As the child increases in age the amount of time he spends in alert, awake activity increases. In the studies described here, there was a clear difference between the state of the newborn and three-month-old infant. Frequent attempts were necessary to keep the newborn awake, while the 3-month-old was able to maintain his alertness by himself.

In addition to the increasing wakefulness of the infant with age, some consideration must be given to the capacities of the infant at integrating sensory phenomenon. Brown[3] has suggested that the newborn can handle only one channel of information at a time, i.e., if he is sucking he cannot also be looking. The infant's early development might be seen as the increasing integration and coordination of information channels. At three months the process seems to be sufficiently advanced so that the infant will alert and orient toward new stimulation, while for the newborn the same stimulation is regarded as an intrusion to be ignored or avoided.[20]

OVERVIEW

At the beginning of this paper two general issues were raised in relation to understanding the behavior of the young infant. The first was the contrast between psychological and biological functioning so that the former could be identified as it emerged from the latter during the infant's development. The second point was the active nature of the adaptational process by which the infant engaged his environment in his development toward more complex levels of behavioral organization.

There appear to be built-in coordinations of the sucking reflex to other behaviors related to the feeding process. A coordinated sequence beginning with stimulation of the cheek, leading to turning of the head, latching onto the nipple, sucking and swallowing is organized by the time the infant is born. In addition, these reflexes can be shown to be coordinations of sub-reflexes, e.g., the suction and expression components of sucking.

Feeding is the single necessary point of engagement between the infant and his environment for the child's biological survival. The prenatal coordination of the feeding pattern permits the newborn to be maximally adaptive to a variety of environmental variations in the source of nutrition. The infant must be capable of responding to a variety of human and artificial nipples if he is to survive. The quick adjustment to many of the feeding contingencies in the research described earlier gives credence to this view of the newborn's flexibility in feeding.

Along with the infant's almost reflexive adaptability, it does appear that he can gain information from his experience and modify his performance in the direction of optimizing food-getting. The alterations in sucking behavior demonstrated during the reported experiments show the capacity of the infant to alter the reflex coordinations in his sucking behavior.

Initially, there is very little engagement of the environment outside of the feeding situation. The infant spends most of his non-feeding time caught up with responding to internal stimulation, leading to either sleep or aroused crying. Attempts to elicit orientation to exteroceptive stimulation unrelated to reflex behavior usually have met with failure except during very brief periods of alert awake activity.[9,22] At the same time attempts to integrate new stimuli into the reflex patterns of the infant as in classical conditioning studies have also met with failure.[20]

As the child ages, however, his engagement with the external environment increases. The initial reflexive reactions to distant stimuli, e.g., pursuit of visual targets, become differentiated through the child's experience with visual inputs including a variety of shapes, sizes, and colors. The differentiations the child's experience allow him to make in these sensory modalities are accompanied by new coordinations between sensory and response systems. Papousek[14] has demonstrated how integrations between auditory stimulation and the feeding responses which were not seen in the first weeks of life appeared towards the end of the first month. The studies reported here have shown how stimulation which was ineffective at inhibiting sucking in the newborn period was very effective at three months of age.

Piaget[15] has described the first two years of life as the period in which the infant must make differentiations within and coordinations between the sensory and motor systems. What has been described above are the initial manifestations of these differentiations and coordinations. The first contacts with the external world serve to both initiate the child's psychological development and to provide the raw material for his cognitive growth.

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Chapter 9

SUCKING AS AN INDEX OF MOTHER-CHILD INTERACTION

Dugal Campbell

This paper takes up the question: can we use sucking as a measure of mother-child interaction? What I have to say falls into four parts: at the start there are some general remarks intended to show that it is worth our while taking up the question; in the middle of the paper there is a description of measures of sucking and their relations with variables likely to have some part in the interactions between mother and child; there is then a short account of two experiments which show that sucking does vary in a manner that can be related to handling; and finally I shall suggest the directions it may be useful to follow in future experiments.

In many fields the progress of experimentation turns upon the selection of some reliable techniques by which the experimenter may show the influence of the variables in which he is interested. These techniques often use effects which, once the main object of experiment, are now so thoroughly understood that they may be used as tools for other purposes. Examples that come to mind are the use of the action potential in the neuron: once the centre of interest for its own sake, now used to elucidate the coding functions of the visual system; and the use of the electroencephalogram to document the phases of the sleep cycle.

Sucking in the human infant has been examined by a substantial number of investigators. Some of them have used sucking in exactly the way I have in mind: for example, non-nutritive sucking has been used as the dependent variable in a number of experiments intended to find out if the human infant can learn (e.g.,[25,32]). The question I have proposed for discussion can therefore be put in a more precise form: is enough now known about sucking to permit its use as a dependent variable in experiments if the variables manipulated by the experimenter are chosen from those which enter into mother-child interactions? The fact that sucking has already been used as a dependent variable is not a sufficient answer to the question because in most studies (on learning, sensory discriminations and the like) non-nutritive sucking has been used as the dependent variable. There are reasons, which I shall go into shortly, for thinking that only nutritive will serve the turn and, in consequence, the work already

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done may not form the basis of a good experimental model of mother-child interactions.

There are a number of reasons why we may wish to look for a measure of mother-infant interactions. First there is now a substantial interest among developmental psychologists in the interactive behaviour of mothers and their children: this interest flows from a variety of intellectual sources but a major impetus to experimental work has been the success of ethologists in recording and manipulating the minutiae of social interactions in a variety of species. They have given an example to psychologists by recording the events of the earliest human interactions (e.g.[28]). Despite the development of an interest in early interactions there are still few techniques for recording infant responses when the mother is handling her infant, in a manner which will yield quantifiable scores (even when one can record what she is doing). In general it is hard to find any act of the infant which can be elicited sufficiently reliably to serve as an outcome of any experimental manipulation so it is not at all surprising that there is a dearth of measures of maternal interventions. Sucking is an act which the infant can and will readily perform; it can be scored in an objective manner yielding intelligible quantities from a record that does not require heroic investments in time or money to obtain. For these reasons it merits investigation as a possible dependent variable in studies of mother-child interaction.

There are two additional reasons for exploring the possibilities offered by sucking; the infant is not only a subject of intervention, he is also an agent in his relations with his mother: the infant, by his initial responses, can influence such processes as the formation of attachments in the mother.[4,29] "Good" and "poor" feeders are often discussed by mothers and sucking may be a measurable aspect of their performance which contributes to the mother's attitude to her child.[9]

Further, because feeding has to be done again and again during the first weeks of life it offers a chance to look into any long-term effects of acts by the mother towards the child (and vice versa); sucking may be a useful dependent variable in such studies and provide data about the cumulative aspects of mother-child interactions. Such cumulative effects, though often taken for granted in discussions of infancy, have yet to be clearly put on record. Thus, in proposing sucking as an index of maternal handling, I am suggesting that we study the feed as a prototype mother-child event. Feeding is the "cockpit of infancy".

The studies which have examined feeding as the prime interaction between mothers and their children during the first days of life have not used sucking as a measure. Kulka, Walter and Fry[24] recorded heart rate and muscle tension simultaneously from mothers and their infants; in their experiment the records of mother and infant tended to show concomitant variations, for example, when the feed was going well muscle tension fell in both members of the pair. Newton[26] used questionnaires to select mothers in studies designed to show that maternal anxieties and handling varied together; her results showed that anxious mothers had more difficulty in dealing with feeds. Recently Thoman has carried out a series of studies in which the independent variable has been handling as carried out by nurses, multiparae and primiparae.[35,36,37] Her results show that handling differs in a con-

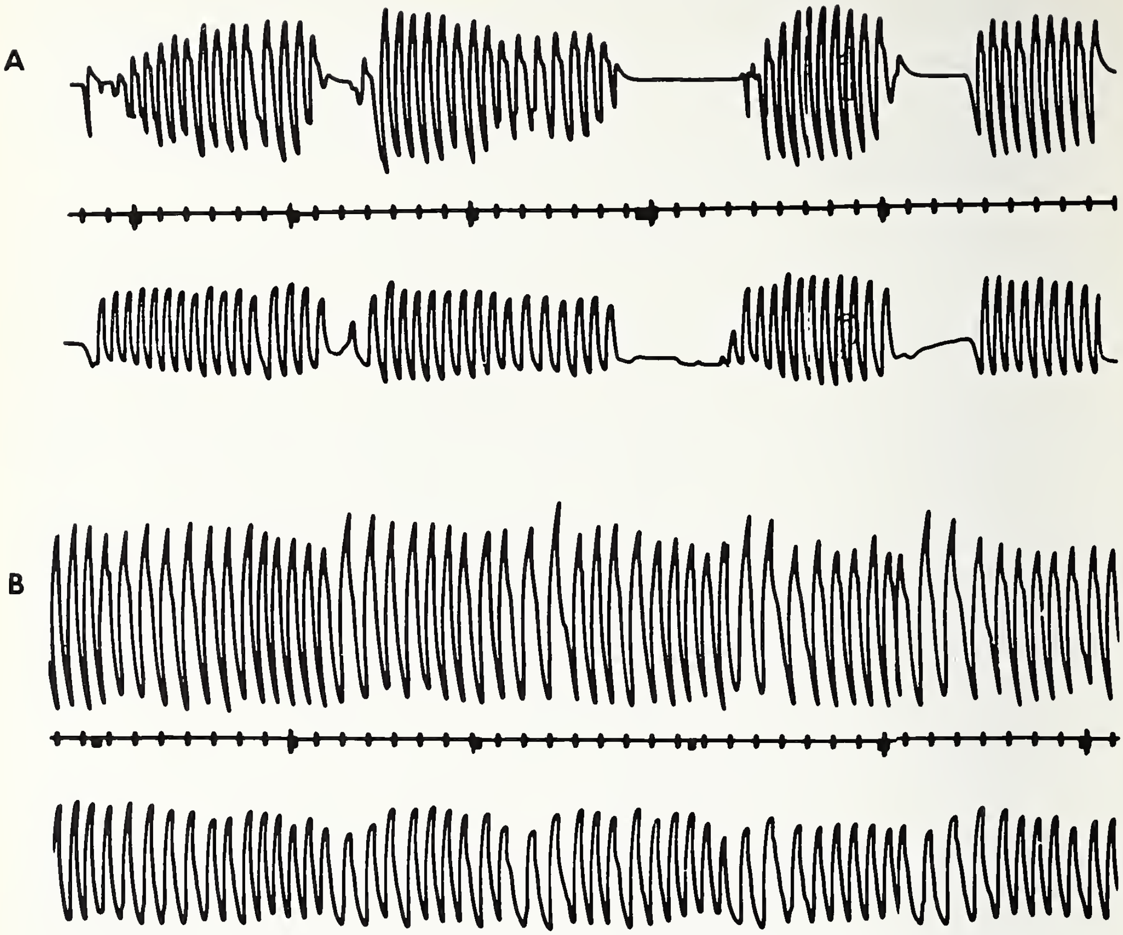

Figure 9–1. Records of non-nutritive sucking (A) and nutritive (rewarded) sucking (B). Both records show suction in the top line and expression below the time markers.

sistent way between these three groups and has an appreciable outcome on the fluid intake of the infants. As in other studies[14] the mothers who have handled babies before manage to get their infants to take more during a feed.

This particular result is one which more clearly than others suggests that sucking may be influenced by maternal handling, and is therefore worth study. In experiments on sucking the variance between individual infants is usually significant. These individual differences may be shown during the first days of life.[23] In addition, fluid intake during the first few days of life has been shown to be correlated with scores of both nutritive and non-nutritive sucking obtained before feeds were instituted.[8] It is probable therefore that mothers who cause their infants' intake to vary do so by an influence upon the sucking of their infants. This supposition is reinforced by one aspect of Thoman's results: the primiparae spent a greater time attempting to feed their infants so that the reduction in intake was due, not to some failure on the mother's part to attend to the child but more probably to some change produced directly in the infant; the argument applies, *a fortiori*, to nurses who produced the greatest intakes and spent the least time feeding.

If we grant that the feed provides a suitable venue for looking at mother-infant interaction and that sucking may be a reliable dependent variable, we have to consider how sucking is to be measured. Sucking is readily elicited, it is easily recorded, the records can be turned into numbers, and finally infants will suck for minutes at a time and so give the experimenter an opportunity to vary the circumstances in a logical way according to his experimental design. Ways of measuring sucking are suggested by the major features of sucking records which can be seen in the figure: the records were taken from one child, aged 3 days, and are best considered as two pairs of records. The upper pair of lines are a record of non-nutritive sucking; the topmost line shows the expression component of sucking and the lower line shows the suction component. Expression is a record of positive pressure exerted on the nipple when it is squeezed by the gums, lips, tongue and palate; these actions are thought to deliver milk in a manner akin to 'stripping' the teat in hand milking a cow. Suction is the record of negative pressure inside the mouth created by dropping the tongue away from the palate.[2] The two sets of acts required to produce these recordings are combined in normal sucking (a feat which adults have difficulty in reproducing). They do not always occur together and on occasion the suction component may drop out when the expression record continues; in my experience the converse is hardly ever seen. This exemplary record shows that sucking occurs in bursts, that the intervals between bursts are all much the same, that the number of sucks in a burst are all much the same, and that within a burst, the individual sucks appear at a steady rate. These regular features of sucking and the fact that they can be readily expressed as quantities are among the reasons for selecting it as a dependent variable and they also suggest simple ways of expressing the pattern in figures.

The method I wish to advocate depends upon two aspects of the record which are among those that control intake and are likely to show the effects of handling. In order to maximize intake an infant must spend time sucking, and while sucking he must either suck at a high rate or he must exert pressure to draw milk from the teat or both. The pressure aspect of sucking I propose to leave aside altogether for the moment; my reason for doing so is that measurement of sucking pressure demands more in the way of apparatus and, in my experience, mothers are likely to be put off their stroke in experiments which replace the usual bottle by a fancy bottle. If an unobtrusive means of measuring pressure can be found then it would be sensible to measure it because in experimental feeds it is closely associated with intake.[22]

The scores that can be used with simple apparatus are *sucking time* and *sucking rate*: sucking time is the amount of time a baby spends actually sucking, and sucking rate is the count of sucks made divided by the sucking time. That is to say, sucking rate is the within-burst rate, exclusive of inter burst intervals. Please notice that this definition of sucking rate differs from another possible score obtained when rate is defined by number of sucks per trial. If rate is defined in terms of trial length it is ambiguous because changes in the score can be due either to an increased time spent sucking or to changes in the speed of sucking within bursts. The definition offered here also has the advantage that even a short sucking burst can give a true rate although it may be the only event in the trial. The evidence now available to us suggests that sucking rate does not vary during a feed;[13] at the end of a feed the baby's sucking time falls off but, when he sucks, the rate is unchanging from start to end. This finding suggests that the baby's control of feeding (i.e. satiation mechanism) is by time spent sucking. It may also be the means by which the mother manipulates intake.

The contrast between the pair of top lines and the pair of lower lines illustrates another well-established fact: the upper pair was produced by a baby sucking on a dry nipple (non-nutritive sucking) and the bottom pair was produced when the nipple delivered a 5% dextrose solution. The delivery of fluid increased the length of the bursts, reduced the interval between bursts and reduced the rate of sucking within a burst. These differences must lead us to consider if both patterns of sucking will serve equally well in experimental studies of mother-child interactions. It would certainly be easier to do such experiments using non-nutritive sucking.

The evidence now available suggests that nutritive sucking is more closely related to what takes place during a feed than is non-nutritive sucking. I mentioned earlier that measures of sucking made during the first day of life correlated with fluid intake during the lying in period. Sucking time, recorded before regular feeds had begun, was positively correlated with fluid intake over four days. This was true *both* of measures of nutritive sucking and non-nutritive sucking. It is no longer true of sucking time scores obtained on the third and fourth days of life when *nutritive* sucking only is correlated with fluid intake.[14] Sucking rate scores, by contrast, do not have any association with intake scores at all, either before feeds begin or when recorded later. These results show that one cannot regard the two kinds of sucking as interchangeable for experimental purposes after regular feeds have been instituted for a few days. Although it is clear that non-nutritive sucking and nutritive sucking are alike in many respects I believe that experiments designed to show maternal influences upon infants must use nutritive sucking as the dependent variable if they are to lead to a grasp of what takes place during real feeds.

In speaking of sucking I have emphasized various possible measures of sucking and their relations to each other and to measures of intake. Moreover, in offering sucking as a dependent variable for experiments upon mother-infant interactions I have spoken of it as an "index": in both instances a complex process is in view and, for purposes of simplification, a single measure of the process may be useful. In effect we look at the process from outside: a change in the index measure, even when it follows directly upon some obvious adjustment in input, does not permit us to infer, without other data, a direct causal relation between input change and output change. The advantage that it gives us is that when changes take place, we are given some hints about what experiments to do.

We can develop experiments more efficiently when we have some notions about the nature of the process we wish to study. One possible way of analysing feeding follows from analysing it as a skill.

A feed is a collaboration of acts carried out by mother and child — a collaboration akin to many others such as ball-room dancing or show jumping. A skill can be defined as a sequence of actions, carried out in an appropriate sequence and controlled both by internal reports about what is being done as well as by external signals about the success of what has been done. In addition many skilled acts depend upon anticipation of what will come next as a means of shaping the motor acts actually executed. When feeding is described in this manner it transfers our attention away from the component actions of the skill to their mode of organisation and to the signals which are exchanged between the actors.

In the case of the mother it is easy to see how feeding resembles other skills: the comparisons between primiparae and multiparae made by Thoman suggest that feeding, like other skills, improves with practice. The primiparae behave unskilfully by carrying out more actions that do not have to do with feeding, breaking into their partners sequence of acts more often and by missing clues necessary to planning their next actions. In these respects their behaviour resembles that of unskilful pilots or nervous pilots who change their plans too often, adjust the controls too much and miss critical signals.[30] The similarity between lack of skill in unpractised pilots and lack of skill in anxious pilots has been forcefully drawn and suggests that one possible line of work would be to compare the details of the feedings performances of calm and nervous mothers with a view to showing how their conduct influences the sucking of their babies.

Treating the infant's contribution to the feed as a skill may seem, at first, less plausible because babies suck well at birth and skills are usually defined as learned acts. However, sucking has many of the features of a skill: a sequence of actions (head turning; mouth opening; expressing; suction; swallowing; breathing) are formed into a smooth performance; relevant signals (nipple features; the delivery of fluid) are detected and alter performance; performance varies in accordance with the mechanisms required (expression or suction) to deliver liquid. Nor can one exclude the possibility of learning in the infant during the first days of life;[27] during these days the time to accept the nipple when it is offered falls, and both sucking time and sucking rate increase.[8]

When one watches women, especially nurses, feed babies the notion of skilled performance is illustrated in two ways: the nurse plys the baby with stimulation to make him suck and she watches closely for signs of satiety, gas, too rapid a flow of fluid, sleep. Thoman has emphasised this last point: ". . . these variations in infant behaviours reflect not only the capacity of the infant to provide cues in the feeding situation but also the capacity of the nurse to respond to cues provided by the infants".[36] During a feed the skills of the mother and child are linked by the signals each sends out and by the response of each partner to the signals sent out by the other.

In a diagram I have attempted to show one side of this two-way traffic by enumerating the stimuli present during a feed which may be effective signals or influences for the infant during the first few days of life. Much of the diagram is self-evident but it helps to make two points: first, that the feed provides the infant with a complex pattern of stimulation and second, that experimental data now goes some way to telling us which kinds of stimulation control sucking. The diagram shows in outline the prin-

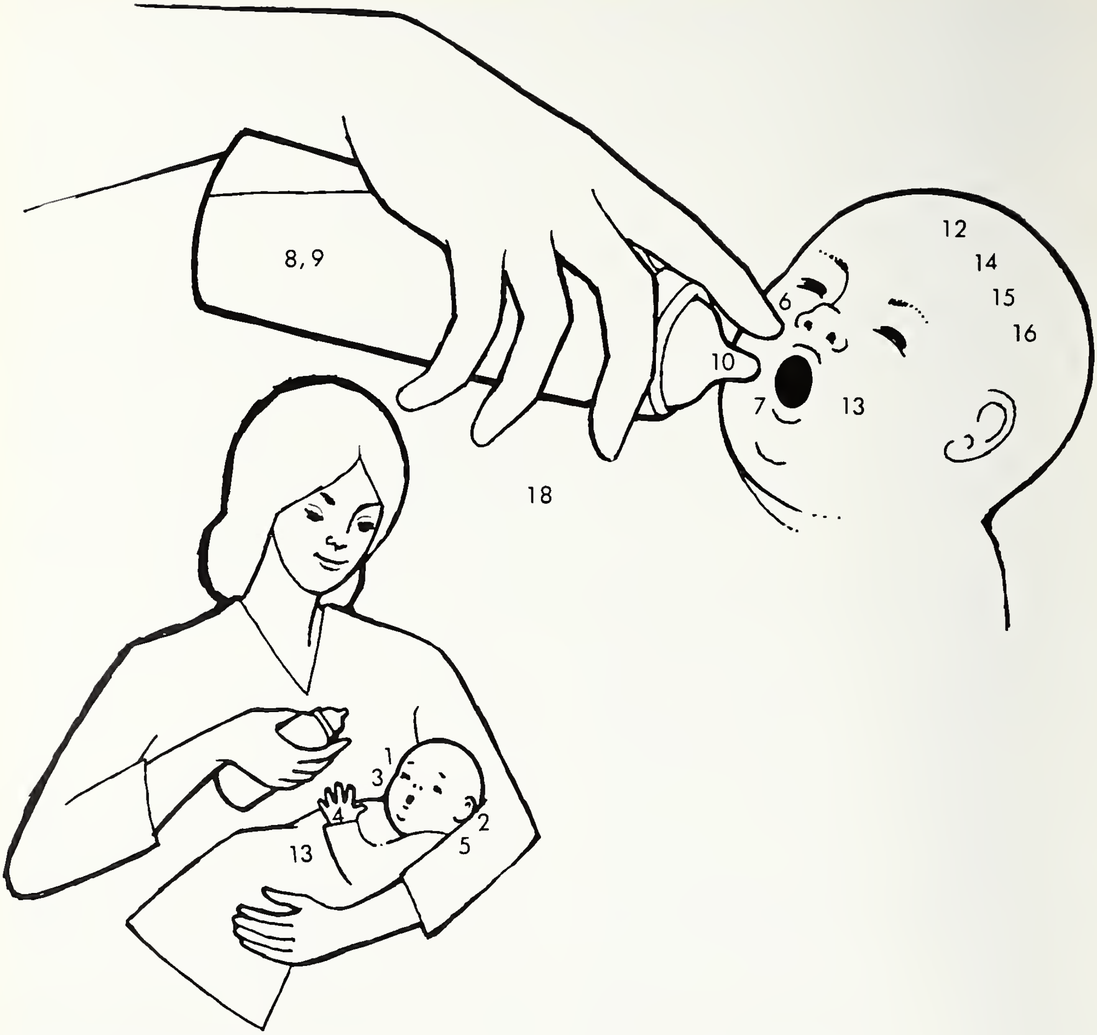

Figure 9–2. Schematic representation of factors impinging on baby during feeds.

Key:

1. Baby sees*
2. Baby hears*
3. Baby smells*
4. Baby feels*
5. Baby is moved*
6. Face touch*
7. Nipple moves in mouth*
8. Fluid qualities
9. Fluid flow*
10. Nipple qualities
11. Hunger*
12. State*
13. Feedback
14. History: immediate*
15. History: remote*
16. Clinical factors

*under mother's control or influence

cipal actors and the numbers entered on the diagrams show the various means of influencing the baby. The numbers marked with an asterisk show the variables that can be controlled by the mother: some must operate immediately in every feed (the baby sees the mother, smells the mother, hears the mother, feels the mother, is moved by the mother); some can be more variable (the rate at which the mother offers the milk; how hungry the baby is when mother picks him up) but even those can be made to vary systematically by some mothers as Ainsworth and Bell[1] have pointed out in their descriptions of feeding patterns. There are few other interpersonal situations in which one person can bring such a barrage of stimuli to bear upon another when they have first been able to prepare the state of the second person. For many of these input channels there is recent experimental work to show that the baby is more appreciative of nuances of the stimulus pattern than he has been given credit for: vestibular stimulation alerts the baby and makes him look round;[21] the infant can distinguish the phonemes of speech;[17] infants fixate upon contours in the first days of life.[31] Thus many of the stimuli manipulated by mother provide inputs to which the infant is attuned.

When the mother puts the bottle in the baby's mouth new means of input are available: movement of the bottle, the fluid in the bottle, rate of flow of the fluid, the nipple. Within the baby too there are controlling stimuli (hunger and state) and past events to have an influence must change some aspect of the baby (clinical history, previous feeds, course of the current feed).

The list of categories shown in the diagram has been entered in a table: each type of stimulation is shown with an indication of what experimental data can tell us about the effects of that stimulation upon non-nutritive and nutritive sucking. The first notation shows if stimulation in a particular mode is known to have some effect upon suck-

STIMULUS AND RESPONSE IN EXPERIMENTS ON SUCKING

	Some Effects		Specific Effects			
	Non-Nutritive	Nutritive	Non-Nutritive		Nutritive	
			Time	Rate	Time	Rate
DISTANT						
1. Baby sees*	---	---	--	--	--	--
2. Baby hears*	Semb & Lipsitt 1968	---	✓	--	--	--
3. Baby smells*	Bronshtein 1958	---	✓	--	--	--
CLOSE						
4. Baby feels*	---	---	--	--	--	--
5. Baby moved*	---	---	--	--	--	--
6. Baby touched (Face)*	---	---	--	--	--	--
INTRA-ORAL						
7. Nipple moved*	---	---	--	--	--	--
8. Fluid (Delivery/taste)	Dubignon 1968b	Dubignon 1969c	✓	✓	✓	0
9. Fluid flow*	---	Sameroff 1968	--	--	✓	0
10. Nipple qualities	Dubignon 1968a	Christiansen 1972	✓	?	✓	✓
INTERNAL						
11. Hunger*	Jensen 1932	Dubignon 1969a	✓	?	✓	0
12. State*	Bridger 1962	Bell 1971	--	--	0	0
13. Feedback	---	---	--	--	--	--
14. History: immediate*	Brown 1972	Kobre 1972	✓	?	?	?
15. History: remote*	---	---	--	--	--	--
16. Clinical history	---	Dubignon 1969b	--	--	✓	0

---	No data	✓	Positive effects
?	Ambiguous data	0	No effects

Figure 9–3. The table lists illustrative experiments to show that the factors shown on figure 2 have an influence upon sucking; generally nutritive sucking rate is not changed by stimulation outside the mouth and nutritive sucking time is.

ing, and the second is designed to show if the effects are specific to either of the two sucking scores I have recommended. A number of points can be drawn from the table: there is no data available on the influence of close stimuli upon sucking; the data about sight and hearing are, with the exception of the studies cited, confusing and, for the moment, one cannot assume that seeing or hearing have an influence upon sucking. By contrast the intraoral stimuli have substantial effects, in particular, upon sucking time when nutritive sucking is examined. No adequate study has yet been done on the cumulative effects of sucking experience and, when experiments have been done in two sessions on the same day[16,33] the later performance does not show an enhancement during the second session.

The results in the two right-hand columns show that sucking time is influenced by a number of variables. Sucking rate is less influenced and changes only when the character of the nipple is altered. Maternal influence is therefore likely to be most clearly seen in measures of sucking time.

The list of variables that may have some part in mother-child interactions shows that there is plenty of scope for experiment; by the same token there are likely to be problems of experimental control if the experiment is done in a real-life setting where it is hard to specify all the relevant features, or to manipulate the variables one by one. Dr. Judith Dubignon and I have done an experiment which illustrates both these points; we made a fairly crude manipulation and examined nutritive sucking in babies who were either held in the arms of an experimenter or laid on their backs in a crib. We did the experiment for two reasons: we wished to find out if "mother", taken in some global sense, has an influence upon sucking during feeds and also because in another experiment on conditioning, we had suspected that babies who were held during the experiment tended to suck less and to go to sleep more readily than those who were not held.

Forty full-term, bottle-fed infants were seen on either the third or the fourth days of life; they were chosen by the following criteria: spontaneous or low-forceps delivery; a labour length of more than 4 hours and less than 24 hours; an estimated gestational age of more than 37 weeks and less than 43 weeks; and exposure to anaesthesia, either general or epidural. These criteria were based on our earlier work[14] which had shown that clinical events and gestational age have an influence upon nutritive sucking; they were designed to assemble a homogenous group of subjects whose histories were all relatively similar with respect to the relevant aspects of their history. Two components of sucking were recorded: expression (the positive pressure on the nipple exerted by the tongue and gums) and suction (the negative pressure generated inside the mouth). Both components of sucking were recorded from a nipple which also delivered 5% Dextrose solution at 38°C. The rate of delivery was 0.5 cc for every 10 sucks (counted by one of the experimenters).

During the hour before a feed each subject was changed and then brought to the laboratory where ECG electrodes were put on the back and chest. The baby was loosely bundled in a carrying blanket: in the held condition the baby was carried in the usual feeding position, i.e. in the crook of the left arm with the baby's head resting against the left side of the chest. In the not-held condition the babies lay supine in a bassinet. The subjects were randomly allocated to the two groups. During both conditions the first experimenter offered the baby the nipple, counted every suck she could see and signalled every 10th suck on the polygraph record by an event mark. A second experimenter monitored the polygraph record and delivered 0.5 cc dextrose to the baby when every 10th suck was marked. In effect we were using an FR 10 schedule of reinforcement. Each subject began with a 2-minute trial during which non-nutritive sucking was recorded; if the baby did not suck at least 30 times in each minute during the baseline trial he was dropped from the study (two babies were excluded for this reason).

The experimental trials followed at 30-second intervals; they consisted of two 120 second trials of rewarded or nutritive sucking. The held and not-held conditions were counter-balanced: one group of subjects was first held and then not-held; the second group was first not-held and then held. The main analyses were done on the last 90 seconds of the recording from each trial; the following scores were used: a sucking count of all the sucks shown on the record; the time spent sucking; and the sucking rate, or count divided by the time. All three scores are available for both components (suction and expression) of sucking. The results of the experiment are shown in Figure 4.

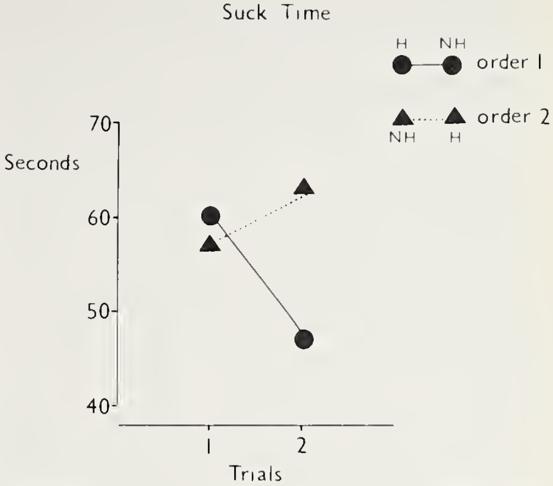

Figure 9–4. Sucking time during the last 90 seconds of trials in which the baby was either held or not held.

The count of sucks and time spent sucking both tell the same story. During the first trial both groups are alike whether held or not held and during the second trial the babies transferred to the arms increased their sucking time and the babies put down reduced their sucking time. The results for the suction components of sucking count and sucking time are statistically significant ($p<.005$); the results for the expression component are not. This difference between the two components is not surprising; we have usually found expersion to be the more robust component of sucking which is less influenced by external stimuli and has a smaller variance. If there is an expression record there may or may not be a suction record; but if there is a suction record there is always an expression record. In this case the more labile suction record shows that holding a baby influences time spent sucking.

Sucking rate increased during the second trial for both groups; the increase is greater in the babies transferred from the bassinet to the arms but is not statistically significant.

The conclusion that handling has an influence upon suction time and suction count was reinforced by an inadvertent circumstance in the experimental arrangements: the babies were run in two series and a different experimenter was 'mother' in the two series. The first 'mother', an enthusiastic hospital nurse, could not bring herself to obey her instructions; she gave the baby a lot of stimulation, e.g. talking to the baby, moving the nipple in the mouth while she held him. The second 'mother' was also a nurse but in addition a graduate student; she imposed the desired experimental control and forbore to do more than support the child. There are significant differences between the suction time and suction count scores of the babies from the two groups; the babies held by the enthusiastic mother who handled the first babies in the series had greater suction time and count scores. The rate scores are the same for both sets of babies. Thus the results that we intended to collect, as well as those that came to us accidentally, show that the time and, consequently, the count aspects of the suction component are influenced by being held. However, it is not clear what variables are actually manipulated in this experiment; several on the list I showed you vary between the two experimental conditions.

In another experiment an attempt was made to capitalize on the work of Thoman and Korner who have looked at feeds given by primiparae and multiparae. These two groups of mothers differ in a number of ways that can be described as less and more skillful. The lesser skill of the primiparous mothers was shown by two results: they took longer to feed their babies and fed them less. Does the handling by the two sorts of mother change the sucking pattern of their infants? This question was examined by comparing the sucking of two groups of babies: one born to and fed by primiparous mothers who had no experience of handling infants and a second born to and fed by multiparous mothers. Earlier attempts to record sucking during feeds using the nipples and apparatus of our earlier work showed that mothers were uneasy in their use of anything but the bottle they were used to. We therefore used an alternative method: we found that a microphone, with the diaphragm removed, gives a satisfactory record of the expression component of sucking if it is secured to the underside of the chin. This method loses the suction component of sucking which may be more sensitive to external stimuli. The babies in this experiment wore a band around the head and the microphone was held against the undersurface of the chin by the band. This arrangement did not appear to inconvenience the babies or to upset the mothers.

The subjects were selected in accordance with the following criteria: spontaneous or low forceps delivery; labour length 4–24 hours; gestational age 38–42 weeks inclusive; birth weight over 2500 gms; an Apgar score at one minute of 7 or more; exposure to anaesthetic and sedation. The children were brought from the nursery to the laboratory, had ECG electrodes put on their back and chest and then bundled in the usual manner for feeding. Each baby had non-nutritive sucking recorded for two minutes. This record was taken for two reasons: first to ensure that the record of sucking taken from the nipple agreed closely with the record obtained from the microphone; and second to check that the sucking performances of the two classes of baby were alike.

Then the mother was brought to the laboratory and asked to feed the baby. The feeding bottle was put on a table at her right hand. One observer made a record of how the mother handled the bottle. She noted on the polygraph when she had it in her hand, when she offered it to the baby, and when she put it down;

her other acts, such as burping the baby were also recorded. A second observer watched the baby and put an event mark on the polygraph chart to show if the baby was visibly sucking when the nipple was in his mouth. Nine multiparae and nine primiparae were seen in this experiment.

The results can be presented under three headings: the babies' performance during non-nutritive sucking; the mothers' actions during the feed; and the babies' sucking during the feed. During the non-nutritive sucking trial there were no significant differences between the babies born to multiparous and primiparous mothers; the infants born to multiparae, in fact, sucked at a slightly higher rate for about the same time and, in consequence, had more sucks during the non-nutritive trial. The correspondence between the expression score, obtained from the nipple used in earlier experiments and the new score obtained from the microphone was good ($r>.93$).

The total duration of the feed was slightly greater when the mother was a multipara. Within the feed there were marked differences in the time during which the mother actively attempted to feed the baby (the multiparae attempted to feed the baby for longer: $p<.01$) and the time during which the nipple was actually in the infant's mouth (the multiparae had it in for longer: $p<.01$). The primiparae presented the bottle more often ($p<.05$), burped the baby more often and spent more time burping the baby ($p<.01$).

The intake of babies fed by primiparae was lower ($p<.05$) and their sucking time was lower ($p<.01$); however, since the nipple was offered to their babies and inserted in their mouths for a shorter time, by the primiparae, the reduced sucking time should be presented as a proportion of the time infants had the chance to suck on the nipple. The two proportions are almost identical (.67 and .68). But there is also a substantial difference in sucking patterns: the babies fed by primiparae had shorter sucking bursts ($p<.01$), a greater number of intervals between bursts ($p<.05$), a greater number of bursts ($p<.05$) and shorter intervals between bursts ($p<.05$). The babies fed by primiparae have a sucking pattern which resembles non-nutritive sucking. There is no difference between the sucking rates in the two groups.

The lower intake of the infants born to primiparae and fed by them appears to be due to two factors: less time to suck on the nipple and a different pattern of sucking once the nipple is presented. Thoman has remarked that the mother dominates the pattern of infant feeding (unlike a nurse who, by contrast, responds to individual differences between babies). The comparison between multiparae and primiparae in this experiment produced results similar to those found by Thoman. One difference should be noted: in her experiments primiparae take longer overall to complete the feed, in mine the multiparae took longer. In all experiments the primiparae spent less time actually feeding the baby and made more changes from feeding to non-feeding and *vice versa*. We can now add that the less skilful primipara breaks into the sucking pattern of her infant more often.

The results of the two experiments show that sucking can be used as an index of variations in handling. They do not tell us what are the critical features of handling which produce alterations in sucking performance. To investigate these I imagine three lines of investigation will be necessary: observations of mothers of different kinds (experienced, inexperienced; strong maternal interests, no maternal interests; confident, anxious) *in vivo* to detect what kinds of stimuli they offer their children; quasi-naturalistic experiments in which the stimuli detected by observation are manipulated during real feeds; and finally laboratory experiments using nutritive sucking as a dependent variable and graded stimuli to elicit graded changes in sucking. I would suspect that, in general, intra-oral stimuli will lead to changes in sucking rate and extra-oral stimuli to changes in sucking time. My own observation of mothers prompts the suggestion that nipple movement, vestibular changes and touching the cheek are all stimuli used by energetic mothers; as far as I know their influence upon sucking time remains unexamined.

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DISCUSSION

KORNER: By choosing the feeding situation to assess the beginning mother-infant interaction, Dr. Campbell has, without a doubt, picked the most cogent and most relevant paradigm. It is the most frequent exchange between mother and newborn during the first weeks of life and, as he indicated, it offers, by virtue of its repetitiveness, a unique chance to study the cumulative effects of early mother-infant interaction. He compared this interaction to a dance in which both partners coordinate their internal and external signals and motor responses to bring about a more or less smooth sequence of actions. I believe, as Dr. Campbell, that the ingredients of this mutual stimulus regulation lie in large part in the synchronization of each partner's tempo and rhythm with that of the other. By studying the infant's sucking patterns in relation to certain maternal actions, Dr. Campbell has captured important aspects of this temporal synchronization.

While non-nutritive sucking may be the preferred measure for the assessment of the infant's response to the onset and offset of stimulation (Bronshtein et al,[1] Haith,[2] Sameroff,[4]) or to gauge the neurological intactness of the infant (Wolff),[9] Dr. Campbell's data has demonstrated clearly that, in the study of the mother-infant interaction, nutritive sucking is the measure of choice. Descriptively, nutritive sucking obviously resembles what actually happens at least during bottle-feeding, much more closely than does non-nutritive sucking. The fact that the two measures correlate significantly with fluid intake on the first day of life and that by the third and fourth day only nutritive sucking is correlated with fluid intake, clearly shows that non-nutritive sucking is an inappropriate measure to use for studying sucking behavior relevant to the feeding situation. This finding also suggests that the infant by the third postnatal day has begun to adapt differentially to the stimulus components experienced in the feeding situation, which implies a primitive form of learning on his part.

Also most interesting are Dr. Campbell's findings regarding the differences in feeding patterns between primiparous and multiparous mothers. It is particularly refreshing when similar results are obtained from two separate laboratories and when this is true, even though different methods are used to assess the problem. Both Dr. Campbell and Thoman et al[5-7] found that primiparous mothers work harder and at the same time succeed less in feeding their babies. In each of the four samples studied, primiparous mothers spent significantly longer in non-feeding activities. In Campbell's sample, they burped the infant more often and longer, and they presented the bottle more frequently. In Thoman's samples, the primiparous mothers provided more stimulation of all kinds, including that they talked more and smiled more at their babies. In Campbell's sample, the multiparae kept the nipple in the baby's mouth longer. In Thoman's breastfeeding sample,[7] the multiparae managed to keep the baby attached to the breast longer. It seems clear from each of the studies that the feedings by primiparous mothers are much more hectic affairs in which the mother engages in an excess of activities not directly related to the feeding.

Thus, judging from the evidence from four separate studies, the parity of the mother significantly affects her feeding pattern. Dr. Campbell, in addition, presented evidence that the infants of primiparous mothers themselves show a different sucking pattern. They have shorter bursts of sucks, a greater number of intervals between sucks, a greater number of bursts and shorter intervals between bursts than the infants of multiparae. The question is whether these differences in sucking pattern are entirely a function of the mother's hectic mode of feeding. While this is undoubtedly true in large part, I wonder whether subtle differences within the infants which have to do with their having been first or later born, may not also be contributory to the mother's style. Little is known about neonatal behavior differences due to maternal parity, largely because most investigators neglect to analyse their data separately for the two groups. When analysis is done, and in those instances where significant differences do emerge, they are always in the same direction: infants of multiparous mothers are more highly aroused. For example, Weller and Bell[8] found that infants of multiparae had significantly higher breathing rates and higher basal skin conduction. In addition, they were more active, even though the difference in this dimension was only of marginal statistical significance. In one of our studies[3] we found that infants of multiparae cried significantly more and were less soothable. Possibly, the fact that multiparous mothers are more frequently delivered without depressant analgesics may account to some extent for the higher arousal levels in their infants. In the case of our study, parity-related length of labor was an important factor: the shorter the maternal labor, the more highly aroused was the infant.

If one entertains the notion that infants of primiparae may have lower arousal levels, and this will have to be shown much more conclusively than it has been to date, then one may view Dr. Campbell's finding that they have shorter sucking bursts, a greater number of intervals between bursts, etc. as an indication that their efforts wane more readily and that their energy ebbs sooner. Perhaps his finding that infants born to multiparae had a somewhat higher rate of non-nutritive sucks also fits into this framework. Even the firstborn's lower milk consumption may in small measure tie in with this.

Of interest in this connection is Thoman's et al[6] finding that when an experienced nurse fed formula to infants of primiparous and multiparous mothers during night feedings, the infants of multiparous mothers required significantly more frequent interruptions in feeding. This again highlights differences inherent in the two groups of infants. While the meaning of this difference is not clear, it may suggest that infants of multiparous mothers, by virtue of higher arousal levels, consume formula faster, requiring more frequent interruptions of feeding for burping, etc.

Thus, while we must conclude from Campbell's and Thoman's studies that primiparous mothers feeding their infants change activity more often and provide more non-feeding stimulation of all kinds, and that they do this out of inexperience and other reasons of their own, they may also in small measure respond, however inefficiently, to something within their infants: they may attempt to rouse them more.

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Brackbill: I was gratified to be asked here, since I am an experimental child psychologist whose focus of interest is not specifically on sucking. So I have, I think, some distance by which to gain objectivity for the research which you reported. Also, I am very gratified to see a tremendous improvement nowadays in the relevance and importance of the work regarding sucking and feeding from the days when I first reviewed the literature on the overwhelmingly popular topic of breast versus bottle feeding.

In those days, for some reason or other, psychologists fooled themselves into thinking that breast versus bottle feeding was some sort of psychological variable, but nobody ever specified what it was. And you people who are currently working on sucking and feeding in early infancy are certainly doing that.

Dr. Brown who has studied sucking as an unconditioned measure and the effect of other variables on unconditioned responding, and Dr. Stanley, who works with sucking in the conditioning paradigm, are very good examples of this. And Dr. Sameroff, too, who used to say that nothing can be conditioned in early infancy, is now less resistant to the prospect that perhaps sucking can be conditioned. Finally Dr. Dugal Campbell's step into the realm of socialization is certainly a great step forward here, too.

I have three comments to make that are common to all these papers. One is on stimulus specification. I think in practically all instances people who focus mostly on a single response or response system such as sucking, sometimes forget to focus as much attention as they might on the *stimulus* characteristics in their experimentation, especially where discrete stimulation is involved. To take orienting, for example, there are two ways of measuring it: one is to wait until the organism is in a quiet state and then to impose a discrete or transient stimulus and measure the response to that stimulus. The other is to wait until the organism is engaged in some sort of ongoing activity and impose the stimulus and measure the cessation of that ongoing activity, as an index of orienting.

The first sort of technique of studying orienting is much more popular, and for experimenters in this area the degrees of specification are fantastic. In using auditory stimuli, experimenters measure not just intensity and frequency, but also rise times and fall times, because all four of these variables have been found by one or more experimenters to make a difference.

Another comment that I would like to make of the papers in general is that I realize that it is easiest to get neonates as subjects. They are a captive audience; they're right there on the ward; and it's not difficult at all to get permission of the mothers if you are even halfway persuasive. But there are a lot of phenomena in any sort of function that show developmental characteristics that obviously cannot be studied in neonates alone. They need to be studied in older infants as well. We all need to be a little more resourceful in our attempts to bring older subjects to the lab.

An alternative, of course, is to use a longitudinal design, getting permission to use subjects while they're still in the hospital, and then ask the mothers to return. Mother are, for some reason unbeknownst to anyone, terribly cooperative in this respect, and they will come back.

Dr. Sameroff's one set of data showing fantastic differences in inhibitory capacity between two and three-month old infants illustrates very nicely the need for age comparisons. And I think that many of the phenomena, that only show themselves here as heuristic possibilities, would seem much more tangible, show many more exciting results, if they were studied in older children as well.

My last point involves orienting and, specifically, cessation of activity as a measure of inhibition, from which we infer orienting. This point involves mostly Drs. Sameroff's and Brown's paper. Dr. Sameroff, I have had for some time a feeling that you had it in for Bronstein et al, and so have many other American experimenters, but in point of fact, Russian babies do differ from American babies in many respects. (Of course, Soviet experimenters differ from American experimenters in many respects, too.)

One of the most compelling differences between Soviet and American babies is in the degree of obstetrical medication that is administered to their mothers. For many years, the rule rather than the exception has been no medication for Russian women. They rely much more on hand-holding and techniques of social support than on meperidine or one of the synthetic local anesthetic agents.

Now I think all of my research on the effects of obstetrical medication on infant outcome has shown that, of all the functions that one can measure in infants, that function which is most vulnerable to the effects of medication is the ability to inhibit responding. That function is the first to show degradation, and it lasts for a long time. As a matter of fact, it lasts well beyond the neonatal period.

This is so sensitive that one can pick up highly significant differences as a function of only 50 milligrams of meperidine. Meperidine is just as effective in producing this dysfunction as an anaesthetic agent itself.

Now, to compare Dr. Brown's and Dr. Sameroff's mother subjects. Dr. Sameroff, your mother subjects are apparently white and middle class? These mothers receive considerably more medication than do Dr. Brown's subject mothers, who were black. In this country, significantly less medication is given black women than white women, according to findings of the Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood.

So it may well be that Dr. Brown's demonstration of that inhibitory capacity in young infants, in confirmation of Bronstein et al., is a function of difference in obstetrical medication at birth. Such medication has a more profound effect than anybody suspected heretofore.

SAMEROFF: I agree that the effects of medication must be taken into account and controlled for. However, the studies that I reported were made while I was in Czechoslovakia, where the level of medication is as low as in the Soviet Union.

The more important factors for studies of attention are clearly the state variables. The studies which have been able to demonstrate cessation of activity as a consequence of stimulation were dealing with alert babies. Dr. Brown reported that her subjects became alert during the process of being carried to the laboratory. Marshall Haith, who is the only other investigator to have achieved sucking cessation, used visual stimuli, thereby requiring an awake infant with eyes open.

As more research is done on orienting behavior in infants, it has become increasingly clear that, if the baby is not awake and alert, orientation does not occur.

J. V. BROWN: I did not include a baby in my studies if it's mother had received more than 100 mg analgesia between one and six hours prior to delivery. The mothers of the babies which were included in the studies had received an average of 50 mg analgesia. In the second study, thirteen out of the 40 mothers had received no analgesia and the amount taken by the other mothers ranged between 25 and 100 mg.

The drug criteria were similar to but slightly more stringent than those used by Anneliese Korner in one of her studies.[1] They were also based on findings reported in the literature which relate the amount of drugs given to mothers during labor to diminished sucking[2,3] and diminished visual attention of the infants.[4] Since I was interested in both sucking and visual behavior, I tried to optimize the effects I might get by using a rather stringent drug criterion.

CAMPBELL: I got interested in this some time after I had read the papers by Brazelton and Stechler about drug effects on infant behavior. I was interested to see how drugs would affect my babies. My comment is that in the hospital where I work, the drug doses differ both from those which seem to be common in say, Boston, and in England, as you might expect. In a Canadian Hospital the levels are just about halfway between those common in the Unites States and Scotland.

In any place you work there is a local tradition. This was brought home to me by one of my colleagues who recently had her first baby: the obstetrician told her it was in the best Scottish tradition—and by that he meant, no drugs at all. They certainly make a difference, and I think every investigator has really got to allow for the sort of cocktail that the anaesthetist and obstetricians habitally use in the place where he works. This is a thing that we really must take account of. It varies from hospital to hospital very markedly.

BRACKBILL: The two variables, state and medication, are orthogonal, really; they can be varied separately and they each make their separate contribution to the rate of response decrement. They are both important, but one is not equivalent to the other nor can one be reduced to the other.

CAMPBELL: I think in principle that is true, but in practice there may be complications about it.

I did a study some time ago in which I was observing infant sleep several days after birth in a group of infants and there were quite marked drug effects on the sleep pattern and on the amount of time the baby spent during sleep states. One result was that demerol, in particular, seemed to have an effect on increasing rapid eye movement (rem) sleep.

Now, I am quite sure it's possible to design experiments so that in fact you can catch the babies in the same state and so forth. If there are drug effects as well, it is much more difficult to do so.

It seems to me that this calls really for very much higher degree of control of the baby's state than is common in fact in most North American investigations. So I think what you say is true, in principle; in practice, it's a difficult thing to do satisfactorily.

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[4] Stechler, G.: Newborn attention as affected by medication during labor. *Science*, 144:315–317, 1964.

BRACKBILL: It's difficult, but I have done it. So it's past the point of mere principle.

STANLEY: Let me comment briefly in defense of animal infants and animal mothers.

First, I think we must hesitate a bit in generalizing about the behavioral differences because our canine mothers, beagle dogs, are typically unmedicated. I have on two occasions explored cesarean delivery of puppies as a means of obtaining puppies that have never sucked a dam's teat. In each case, the investigator performing the cesarean used so much anesthetic on the dam that the puppies were rendered useless for our purposes. Drugs administered to the dam profoundly suppressed the puppies' behavior.

Opposite to a state of behavioral suppression is a state of behavioral arousal. In our research on learning and motivation in neonatal dogs, we go to great lengths to assure that our subjects will be in a suitable state of behavioral arousal when the performance is being recorded. Prior to a learning or testing trial or session, we typically stroke the puppy vigorously down its back to assure a high level of responding. Whenever feasible the puppy is stimulated to urinate and defecate before the back stimulation. Also, we try to terminate learning or testing sessions before vigorous and active behavior has ceased. Such repeated and relatively long term control over the behavioral state of research subjects is difficult, if not impossible, on ethical and other grounds in human infants. The range of behavioral phenomena available for study in human subjects, therefore, must be less than in appropriately prepared and controlled animal subjects.

DR. STEINER: I am concerned about generalizations in use of the terms "swallow", "sucking" and "mastication". Sucking differs in an infant and an adult. The infant has two compartments in his mouth: one between the tongue and palate and the other between the lips and the floor of the oral cavity, while the tongue is pressed against the palate.

These two compartments are more conspicuously distinguished in the adult.

DR. BOSMA: During infantile suckle feeding, the bolus enters a single oral chamber. This chamber is paramedian, between the tongue and palate, bounded anteriorly by the tongue and lips enclosing the nipple and posteriorly by the apposed muscular palate and pharyngeal part of tongue. There is a second, accessory, bolus accumulation area in the valleculae, bounded by the tongue, the epiglottis and the muscular palate and palatopharyngeal folds.* The bolus is displaced from the midline as it passes around the epiglottis.

As part of human oral maturation, the mouth acquires an open chamber, at rest, between the body of tongue and palate. This chamber is separated from the pharyngeal airway by the stably apposed muscular palate and tongue. It is bounded by the checks and the inconstantly apposed lips. The tip and margin of the tongue are variably apposed to the maxillary teeth and gingiva.

During mastication, an additional

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*Bosma, J.F., Truby, H.M., and Lind, J.: Cry motions of the newborn infant. *Acta Paediat. Scan.*, Supplem. 163:61–92, 1966.

chamber is formed on either side, about the approximating molars.

Dr. Steiner: This masticatory chamber reaches its greatest dimension in the rodent and in certain other mammals in the form of a cheek pouch.

Dr. Bosma: The masticated bolus is brought to the paramedian swallow-preparatory position by a separate maneuver.

Steiner: As I recall the physiology of the lactating breast, milk is only partly sucked out by the infant's action. The breast actively expels milk during breast feeding.

Bosma: G. H. Schmidt[1]. and B.S. Cross[2] have recently reviewed the mechanisms of lactation particularly in experimental animals. In species such as the goat and sheep, which have a large storage area in the ducts, cisterns and teats, negative pressure of sucking may supplement the mechanical massage of the expression gestures. But milk ejection from the mammary alveoli is effected by a separate neurohormonal reflex. The afferent limb of this reflex is traceable from the mammary exteroceptors, concentrated about the teat, through the brain stem reticular formation, the nonspecific thalamic nuclei and into the thalamo-cortical circuits. The final link in this neural system is the hypothalamic stimulation of the posterior pituitary to release oxytocin, which causes contraction of the myoepithelial cells in the periphery of the alveoli. Milk obtaining incident to a particular sucking action is the resultant of extraction and expulsion from the storage areas adjacent to the teat, of oxytocin-stimulated expression from the alveoli, and of current cellular elaboration and release of milk.

An additional maternal mechanism involved in the feeding symbiosis is that of nipple erection. When the nipple of the human breast has been stimulated into an erection, it acts like the piston in a pump, being moved into and out of the breast, thus facilitating the explusion of milk from the lacteal pool immediately surrounding the base of the nipple. This component mechanism is often omitted in studies of breast feeding.

These physiologically complex, psychologically modulated, mechanisms make the lactating mammal remarkably different from the nursing bottle.

Nowlis: Both Dr. Sameroff and Dr. Campbell have reported that the negative pressure created in the infant's mouth during sucking is a more labile and easily disrupted component of the sucking response than is the compression of the nipple by the mouth. Because I am using a relatively rigid and precisely shaped nipple in studying the configuration of sucking movements in the newborn, I have become highly sensitized to the very slight methodological variations which can lead to a change in the recording of a given component of sucking. For a vacuum to be created in the infant's mouth, it is necessary that the lips form a tight seal around the nipple, and, particularly with an artificial nipple, the formation of such a seal is highly dependent on the shape and position of the nipple within the mouth. What this means, I suspect, —and this does not necessarily make the phenomenon any less interesting—is that the fact of a compression movement being recorded in the absence of a recorded negative pressure in the mouth may have as much to do with the configuration of the lips with respect to the nipple as it does with any changes in the size of the oral cavity. Those gross oral movements, then, which contribute to the negative pressure within the mouth, may still be operating in the absence of recordable negative pressure.

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[1] Schmidt, G.H.: *Biology of Lactation*, G. W. Salisbury, San Francisco, W.H. Freeman, 1971, pp. 118–136.

[2] Cross, B.A.: In, *Milk: The Mammary Gland and its Secretion.* S.K. Kon and A.T. Cowie (Eds.) New York, Academic Press, 1961, Vol. 1, Ch. 6, pp. 229–277.

Chapter 10

IMPORTANCE OF PHARYNGEAL FEEDBACK ON THE INTEGRATION OF REFLEX DEGLUTITION IN NEWBORN ANIMALS

TADAAKI SUMI, M.D.

INTRODUCTION

The swallowing performance is an intricate coordination of the intrinsic and extrinsic pharyngeal musculature. Its fundamental mechanism is a reflex initiated by sensory impulses from various receptors in the pharynx.[4] The motoneurons partaking in this action are scattered from C3 to the mesencephalon and are controlled by a "swallowing center" in an orderly pattern of excitation and inhibition specific to this organization.[5]

My question was whether this orderly pattern is demonstrable in the newborn as well as in the adult mammal. If not, then, how does it change postnatally and what mechanisms may be involved in such a change? This chapter reviews some of my earlier[19] and more recent neurophysiological investigations on the nature and postnatal development of reflex swallowing in kittens and rabbit pups. Particular attention is given to the role of pharyngeal feedback upon integration of the swallowing reflex in the immature animal. These observations and interpretations are related to some clinical descriptions of swallow incoordination and other forms of dysphagia encountered in human infants.

METHODS

All these experiments were performed in kittens and rabbit pups of various postnatal ages and in adults. Most of the studies were in animals of less than 21 postnatal days. The animals were decerebrated at midcollicular level under light ether anesthesia which was discontinued immediately after the decerebration. In each case adequate ventilation of the animal was secured via tracheotomy. Single fibers of the motor and sensory nerves participating directly or indirectly in swallowing were carried out by splitting their stump, using a pair of watchmaker's forceps under microscopic control. These included the hypoglossal, mylohyoid, accessory, phrenic and superior laryngeal nerves. The nerve activity was fed into a R-C coupled amplifier, displayed on a dual beam oscilloscope and recorded on film by a kymographic camera. The other lead was sewn tightly around the wound in the neck and grounded. The geniohyoideus or mylohyoideus EMG or mechanical deflection of the larynx associated with swallowing was also recorded simultaneously with the nerve activity.

Swallowing was produced either by squirting 1–2cc of water into the oropharynx or by stimulation of the superior laryngeal nerve (SLN) with trains of repetitive electrical pulses. Artificial respiration was employed whenever spontaneous respiratory movements were arrested by intravenous injections of gallamine triethiodide (Flaxedil:$^{(R)}$ 3mg/kg). One per cent lidocaine hydrochloride (Xylocaine)$^{(R)}$ dissolved in water was applied topically, when required, to the mucosal surface of the pharynx.

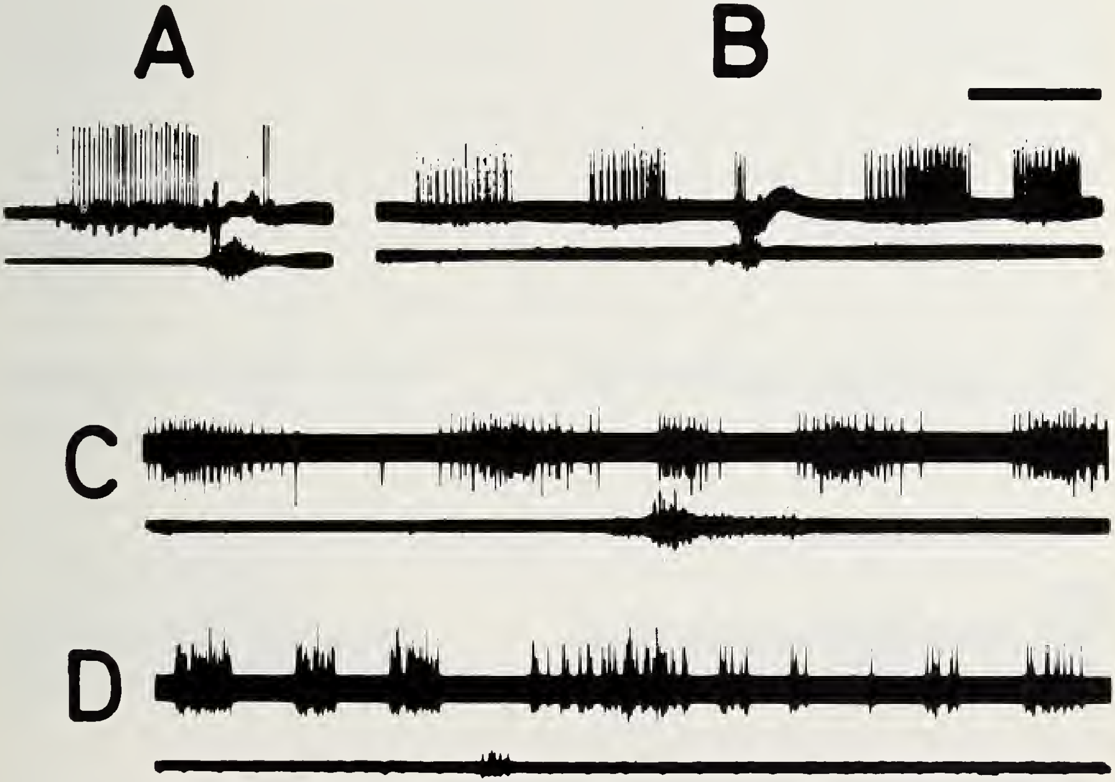

Figure 10–1. Activity in the accessory (A) and phrenic (B) nerve fibers and in the inspiratory (C) and expiratory (D) intercostal muscular units during swallowing induced by a squirt of water into the oropharynx of kitten. Lower traces are geniohyoideus EMG. B, C, and D) demonstrate spontaneous respiratory volleys and the changes interposed in phase with swallowing. Time bar : 1 sec.

RESULTS AND DISCUSSION

In kittens and rabbit pups, swallow-associated activity was found in many motor fibers of the nerves sampled. Corresponding burst activity was also found in motor fibers of the phrenic and intercostal nerves (Fig. 10–1), though these muscles seemingly have no direct participation in the act of swallowing. This activity varied a great deal between fibers and even in the same fiber at differ-

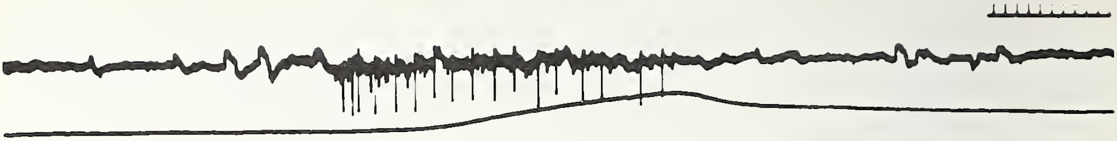

Figure 10–2. Burst activity of mylohyoideus motoneurons in adult rabbit during swallowing elicited by a squirt of water into the oropharynx. Rostral movement of the larynx associated with swallowing is demonstrated by upward deflection in the lower trace. Time: 0.01 sec. divisions.

ent times. In contrast, swallow-related activity in adult animals was restricted almost entirely to the muscles that participated directly in the swallowing act. The motor participation in the mylohyoid nerve is shown in Figure 10–2, and in hypoglossal nerve in Figures 10–4, 10–5, and 10–6. The swallow-related activation in these nerves was fairly constant in mode of discharge in each fiber and during each discharge in the same fiber.

The pattern of motor activity in the hypoglossal nerve fibers in kittens dur-

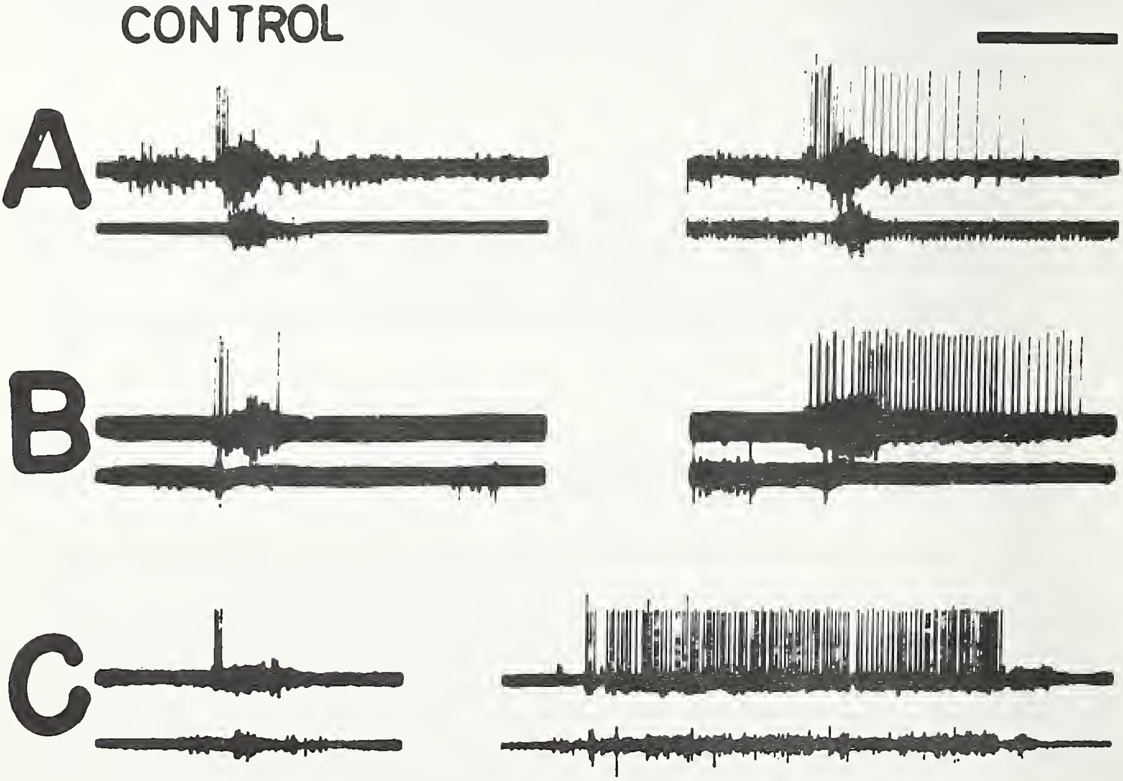

Figure 10–3. Alterations in the activity patterns of the hypoglossal nerve fibers during swallowing in the kitten under different conditions (right column). A : light asphyxia, B: squirting a large amount of water, C : mechanical touch to the pharyngeal surface. Control responses were obtained by squirting a small amount of water into the oropharynx of the animal while it was breathing quietly. Upper trace: motor activity of the hypoglossal nerve fiber. Lower trace : activity of the geniohyoid muscle. Time bar : 1 sec. (Cited from Sumi[19]).

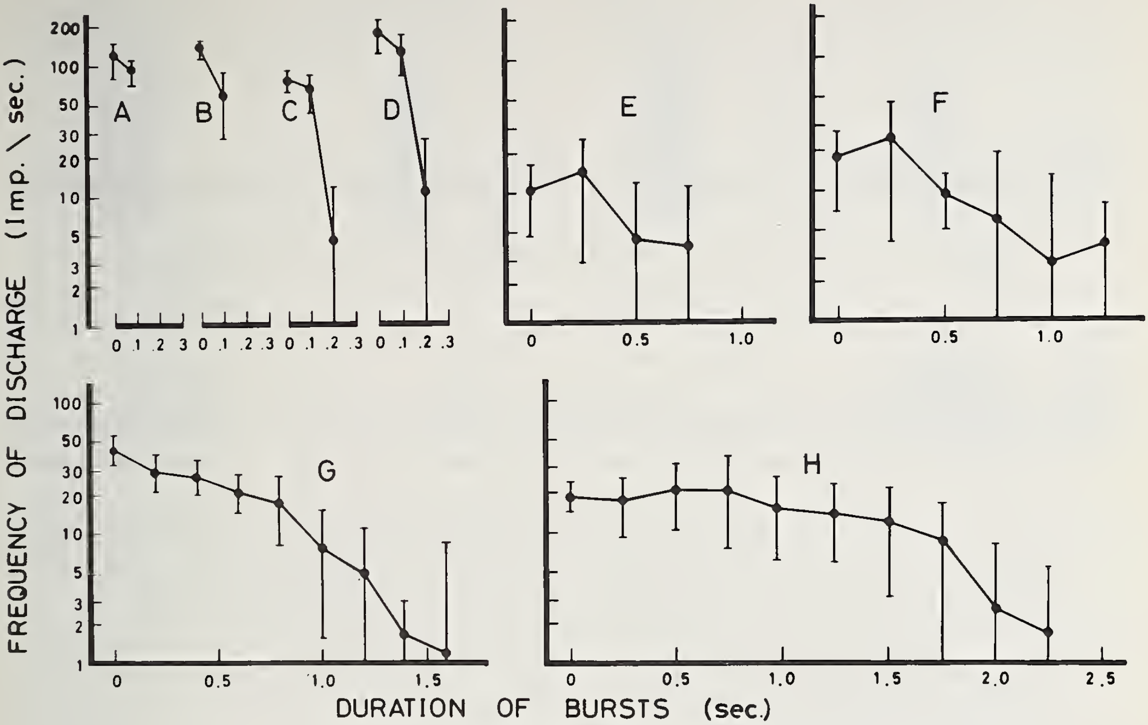

Figure 10–4. Comparison in the discharge frequency and duration of the hypoglossal nerve fibers during burst activity of swallowing between adult cats (A, B, C and D) and kittens (D, F, G and H). Swallowing was induced by squirting a small amount of water into the oropharynx. The frequency (ordinate) calculated from the spikes discharged during each 0.1, 0.2 or 0.25 sec. was plotted against the time (abscissa) after the onset of the burst activities. The height of each vertical bar represents twice the standard deviation of the mean frequency of different burst for the identical unit. (Cited from Sumi[19]).

ing swallowing elicited by a squirt of water into the oropharynx is profoundly altered by associated circumstance, such as light asphyxia, squirting a large amount of water, or mechanical touch to the mucosa as compared to the control when the animal was breathing quietly (Fig. 10–3). Activity of the geniohyoid muscle, which is the first to become active during swallowing, is recorded in each lower trace. In contrast, the mode of swallow-related discharge in the hypoglossal nerve fibers of the adult cat is very stable, and the alteration shown in this figure could scarcely be produced by imposing these abnormal circumstances.

Differences in stability of the swallowing discharge between kittens and adult cats are compared in Fig. 10–4, in which the mean mode of discharge is determined statistically from many samples of analogous discharges in the hypoglossal nerve fibers elicited by a squirt of water. The swallowing discharges of four representative units in adults, indicated by A-D, show a high order of stability with durations ranging from 0.15 to 0.3 sec. and a slight range of variation from the mean. But in the kitten, E-H, the units demonstrate a much longer duration and a wider range of variation in the discharge frequency of impulses. As kittens mature, the modes of

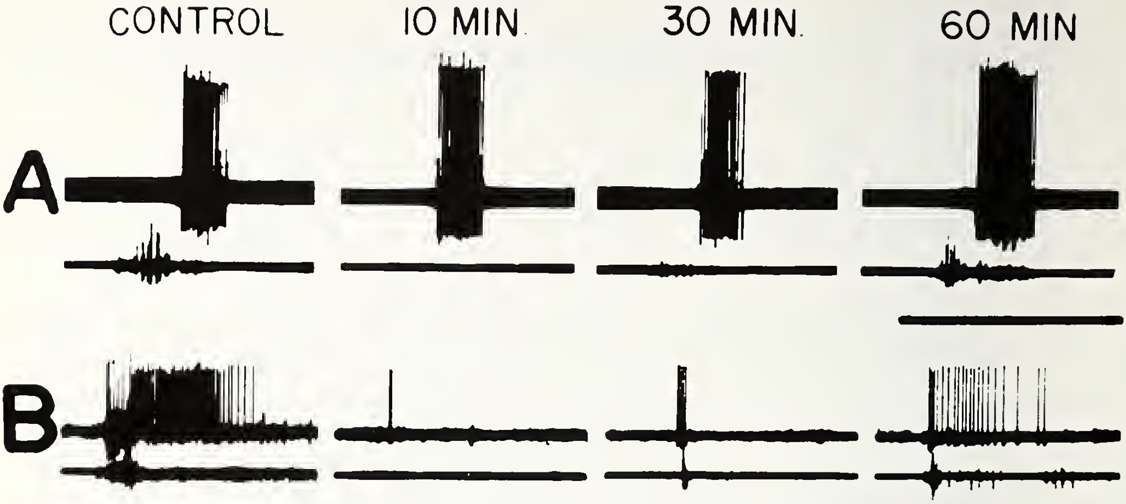

Figure 10–5. Contrasting examples of the effects of motoparalysis on the "swallowing discharge" in the cat (A) and kitten (B). Each burst of swallowing was elicited by squirting water into the oropharynx. After motoparalysis, patterns of the discharge in the hypoglossal nerve fiber (upper traces) are unmodified in (A), but are conspicuously diminished in (B) both in the number of spikes and in the discharge duration. Responses of the geniohyoid muscle (lower traces) associated with swallowing are similarly diminished recovering gradually with this observation period. Time bar : 1 sec. (Cited from Sumi[19]).

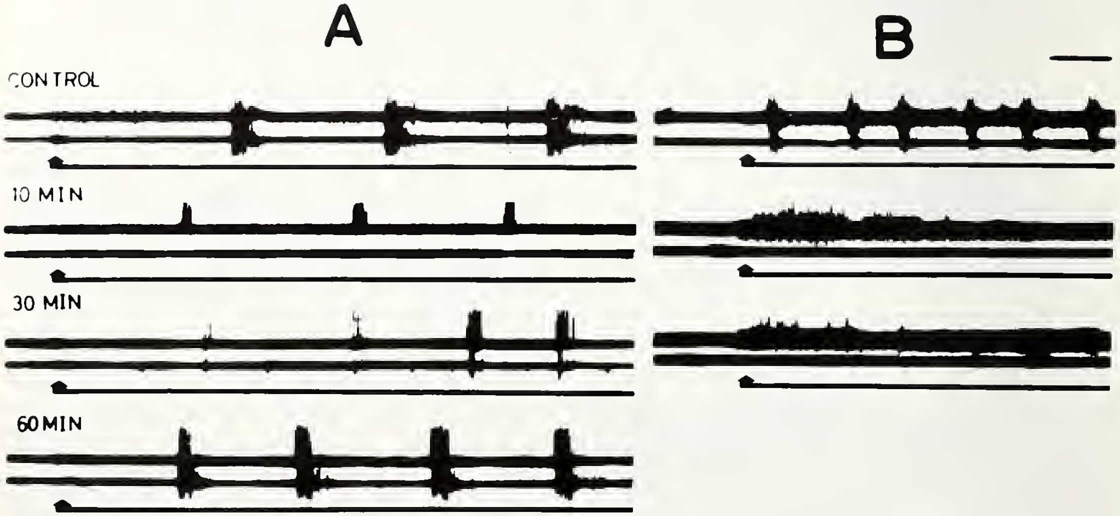

Figure 10–6. Effects of motoparalysis on the responses of the hypoglossal nerve fiber to tetanic stimulation of the superior laryngeal nerve in the adult cat (A) and kitten (B). There is no essential change in the gross schedule of swallow discharges in the nerve (upper trace) in the mature cat. But the repetition schedule is disrupted in the kitten, and replaced by episodes of prolonged firing, shown in the tracing at 10 minutes after administration of gallamine. Activities in the geniohyoid muscle (lower traces) in A and B are similarly inhibited by motoparalysis. Lines with arrows pointing upward indicate the timing and duration of the tetanic stimulation. Time bar : 1 sec. (Cited from Sumi[19]).

discharge gradually approximate those of the adult, attaining full maturity at about postnatal month 3.

Figure 10–5 shows the effect of motoparalysis on the motor discharge of swallowing in the hypoglossal nerve fiber. In the adult (A), there is no notable modification in the mode of discharge of the nerve fiber during motoparalysis by intravenous administration of gallamine. But motoparalysis occasions profound alterations in the response of kittens (B). After motoparalysis, a squirt of water into the oropharynx elicits few or no action potentials, reflecting complete abolition of the act of swallowing. The reflexly elicited swallow is recovered slowly but completely.

As shown in Fig. 10–6, when reflex swallowing was produced by tetanic electrical stimulation of SLN, the effects of motoparalysis were comparable to those produced in the case of squirting water into the oropharynx in the adults. Distinctively in the kitten, the periodic bursts of swallow-related discharges in the hypoglossal nerve were replaced by a continuous train of impulses, especially during the initial stage of the electrical stimulation. The motor impulses appear to follow each afferent stimulus. The geniohyoid muscle remains inactive throughout the period. Again, recovery to the control pattern of reflexly elicited swallow occurred gradually.

These effects of motoparalysis upon the hypoglossal neurogram of swallow reflexly elicited in kittens by squirting water into the oropharynx or by electrical stimulation of SLN changed progressively into mature pattern.This change was accomplished at approximately 3 postnatal months. Though we do not yet have conclusive evidence in the immature animal, we attribute these effects of gallamine to neuromuscular blocking, and not to some direct effect upon the central neurons in the brain stem participating to the integration of swallowing, since gallamine has been shown to effectively excite medullary neurons for only a few minutes, even when applied electrophoretically to their immediate site [15].

These studies make it possible to discriminate the afferent discharges which elicit swallow, designated "primary" from the afferent discharges which are elicited from the swallowing action, designated "secondary". In the immature kitten treated with gallamine, the primary afferent discharges from mucosal receptors in the pharynx are preserved. But the secondary afferent discharges elicited from receptors in mucosa as well as in deeper structures are abolished [18]. The secondary afferent discharges during a reflexly elicited swallow are numerous and heterogeneous.

Figure 10–7 demonstrates the large number and variety of afferent discharges in SLN single fibers elicited by the act of swallowing, in comparison with the relative paucity of impulses in the single efferent fibers of the mylohyoid and the hypoglossal nerves. It is appropriate to postulate that the secondary afferents of swallowing provide feedback requisite for the achievement of adequate swallowing in the mammal which is immature in this neurological organization. Without these afferents, the motor discharge characteristic of swallowing cannot be accomplished in the medullary swallowing center.

Of the secondary afferent discharges mentioned, those initiated from receptors in deeper structures seem to be the more effective in achieving competent swallow performance. When only the pharyngeal mucosa was anesthetized, in

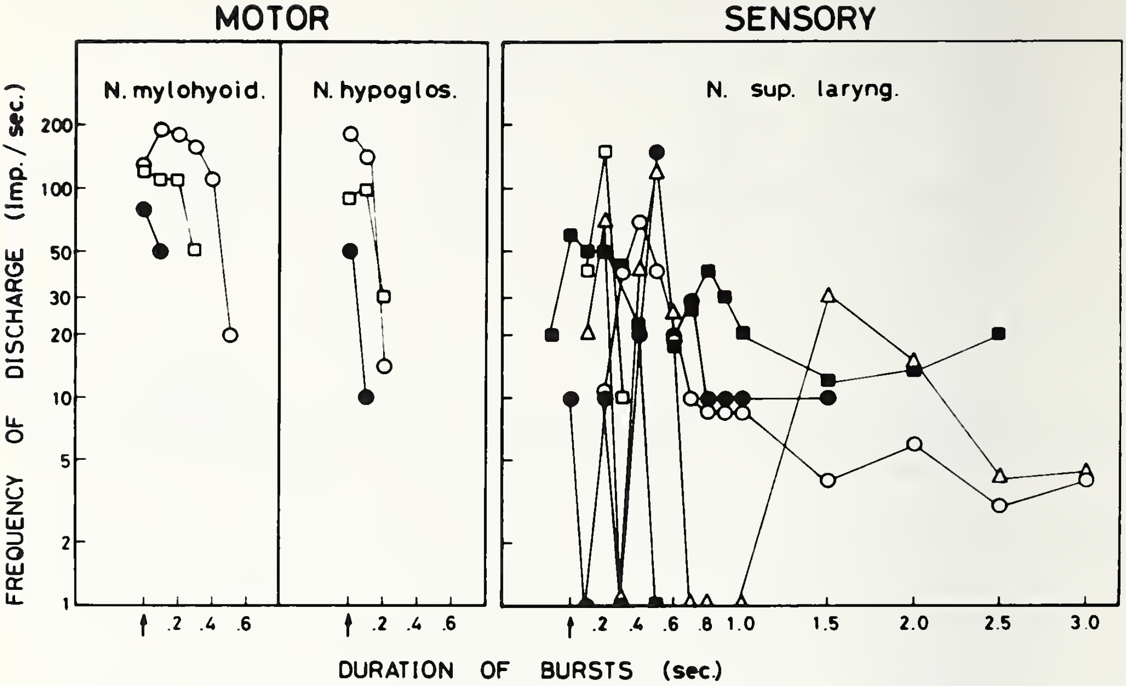

Figure 10–7. Contrasting features in the motor and sensory neurogram of reflex swallow of adult cats. Each symbol represents a different unit. Arrows indicate beginning of discharges.

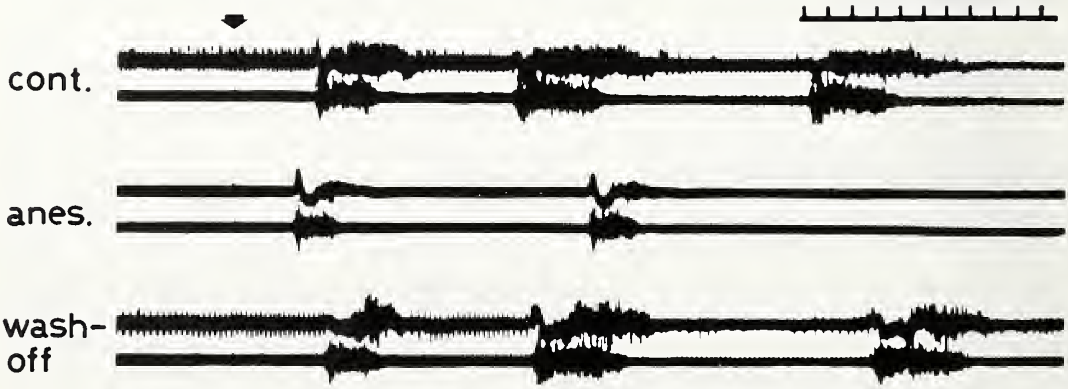

Figure 10–8. Effects of pharyngeal mucosal anesthesia on swallow-related activity of sensory (SLN: upper trace) and motor (Mylohyoid EMG: lower trace) units in an 18 day old rabbit. Swallowing was induced by tetanic electrical stimulation of the contralateral SLN; onset shown by arrow. The middle record was obtained 5 min. after application of lidocaine. The lower record 5 min. after the lidocaine was removed by washing. There is a clear alteration and reversibility in the patterns of activity in this experimental circumstance. In Figures 10–8 and 10–9, sensory nerve fibers from mucosal receptors were exclusively selected by dissection. Time : 0.1 sec. divisions.

rabbit pups, swallowing could occur but the action was incomplete, with a marked reduction in the motor discharge. Figure 10–8 clearly shows swallow-associated burst discharges in both the afferent fibers of SLN and in the mylohyoid muscle during stimulation of the contralateral SLN. After the mucosa of the pharynx was completely anesthetized, no afferent discharge was elicited during swallowing, whereas the motor activity was abbreviated by approximately one half of its duration prior to anesthesia. The continuous train of motor impulses elicited as an effect of SLN stimulation after motoparalysis (Fig. 10–6B) did not occur following SLN stimulation after mucosal anesthesia.

It should be emphasized again that this reduction in swallow-related efferent discharges following mucosal anesthesia is absent or minimally evident in adult rabbits, even though afferent discharges from mucosal receptors are abolished entirely. These differences of the mature rabbit are seen in Figure 10–9. The afferent nerve fibers that could be excited by touch to the mucosa (A), also displayed a burst discharge associated with swallowing (B). After anesthesia of the mucosa, with complete abolition of the activity in the afferent fibers, the swallow-associated burst activity in the mylohyoid muscles was unchanged (C).

It may be inferred, therefore, that the secondary, swallow-elicited afferent discharges which are initiated from mucosal receptors are effective in evoking motor activity in the later stage of swallowing, whereas those which are initiated from receptors in deeper structures may be effective for the activity in the initial stage of swallowing or, perhaps, for the establishment of the characteristic excitation of the medullary swallowing center. Such feedback would have an almost essential role for achievement of competent swallowing when the animal is immature. But it would be less essential as it becomes mature. In the mature animal, the medullary swallowing center has at-

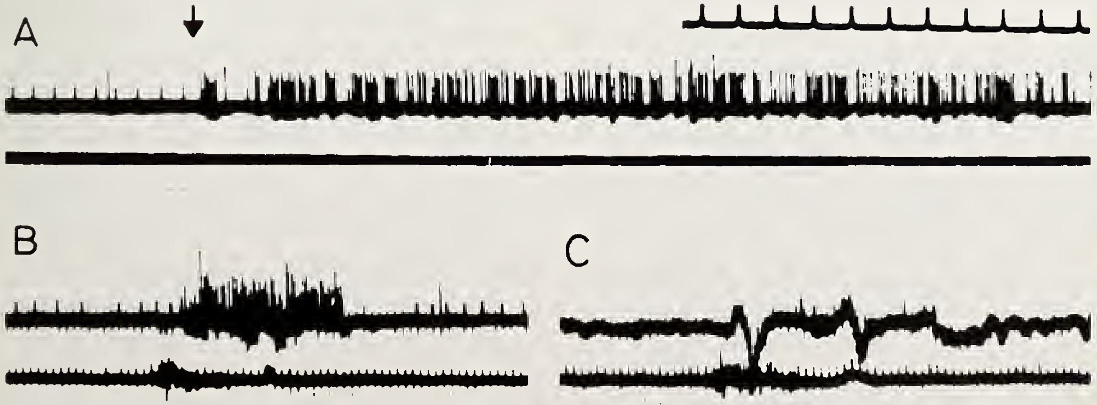

Figure 10–9. Activity in sensory fibers of SLN (upper trace) and in mylohyoideus EMG (lower trace), adult rabbit. Time : 0.1 sec. divisions.

A. In response to continuous touch (onset at arrow)
B. During swallow
C. After mucosal anesthesia

tained its functional organization, and feedback from the periphery is no longer requisite for full competence. In other words, when the primary swallow-eliciting afferent impulses from the pharynx achieve threshold at the medullary center of the mature mammal, its initiation and government of the swallow coordination proceeds in usual pattern. Autonomy of the swallow coordination from pharyngeal feedback is assumed to be progressively achieved during prenatal and early postnatal development through frequent repetition of the act of swallowing during feeding.

Clinical Applications

These demonstrations of differential liability of the immature swallowing organization to disruption may be applicable to clinical care. The risks of local pharyngeal anesthesia and of local touch in young infants are familiarly recognized in pediatrics. Impairment of pharyngeal function is also recognized as a particular risk or liability incident to the administration of curare or other motoparalytic agents.

This immaturity of the swallowing coordination is of particular relevance to impairments in the sequence of swallowing and other forms of dysphagia in human infants. Clinical studies may demonstrate appropriate cause of these disorders, such as malformation in the oral and pharyngeal structures, or neurologic or muscular abnormality.[2,9] In some dysphagic infants, however, there is no discernible etiology or mechanism of the dysphagia other than immaturity of the infant.[1,6,7,10,11,13] In the cases in which the disorder of swallowing is temporary, disappearing in 2–4 postnatal weeks, it is presumed to be related to delayed maturation of the swallowing coordination.[7] The results of this study suggest that in addition to this immaturity, or as an element in it, there may be a deficiency of the secondary type of sensory feedback incident to the swallow performance.

That the vagi are more important for coordination of respiratory activity in the newborn than in adult[16] affords us a notable analogy, for deglutition and respiration are closely interlocked with each other in their operation, regulatory organization and phylogenetic and ontogenetic development.[12,14] It is also of interest that reflex swallowing requires much longer postnatal maturation than that of spinal motoneurons,[8] of the two-neuron spinal reflex[17] or of the brain stem masseteric and digastric reflexes,[3] which attain mature pattern by 3–4 postnatal weeks. This would imply a greater complexity in the neuronal organization of swallowing.

It is known that, in the young infant, swallowing or stimulation to the oral, laryngeal and pharyngeal areas readily give rise to excitatory and/or inhibitory influences upon a diversity of somatic and autonomic functions, as compared with the adult. When, furthermore, the subject is depressed during general anesthesia or during respiratory or circulatory deficiency, oropharyngeal stimulation to elicit swallowing, or any manipulations of the structures concerned, may elicit a generalized dysfunction, which may be critical. On these occasions, as mentioned previously, the afferent invasion may overrule the incoordinated regulatory organization of these functions, which are immature and/or otherwise impaired.

SUMMARY

The performance of pharyngeal swallow, as evaluated by single fiber recordings from sensory and motor nerves, was compared at postnatal ages from 4 hours to physical maturity of cats and rabbits. Swallow elicited by a squirt of water or by SLN stimulation was differently modified at different postnatal ages by asphyxia, by gallamine motoparalysis, or by topical anesthesia of the pharyngeal mucosa. Motoparalysis of the neurologically immature animal resulted in marked disruption of the elicitation and of the motor discharge of swallow. Mucosal anesthesia caused less disruption. These phenomena of immaturity diminished progressively. Neurological maturity of swallow elicitation and motor performance was usually achieved at 3 postnatal months.

The disruption of the immature swallow organization incident to the administration of gallamine is inferred to be an effect of deficiency of the afferent feedback generated by the swallow performance.

The significance of the neurological immaturity of the swallow performance and, particularly, its liability to disruption is applied by inference to clinical problems of dysphagia in infancy.

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DISCUSSION

DR. BOSMA: There are two broad scale implications from Dr. Sumi's work which are apropos of infant pediatrics and neurology. One is that the infant's swallow performance seems to be dependent upon local sensory input, at least from the superior laryngeal nerve. Accordingly, we are warned to be careful about anesthesia of the infant's pharynx; this is already a tradition in pediatrics.

Dr. Sumi has also shown the peculiar liability of an infant mammal to distortion of pharyngeal swallow, following the administration of Flaxidil. In clinical work we are aware that we must be careful of Flaxidil and of comparable peripheral paralytic agents, because of risk of disability of the pharynx. But note that the pattern of motor distortion which was demonstrated as an effect of Flaxidil closely simulates laryngospasm, which may be a major crisis in a young infant.

KENNY: Do you think the bursts you are seeing in single fibers of the hypoglossal nerve which are asynchronous with pharyngeal swallow could reflect a maturation of tongue movements secondary to swallow, rather than maturation of swallow itself? In other words, I am thinking that the tongue itself, with sucking experience, is maturing and fitting into an engram for swallow. This, in turn, is producing the synchrony observed in the mature animal, rather than an actual maturation of the pharyngeal phase of swallow, as may have been implied.

SUMI: The asynchronous firing in single fibers of the hypoglossal nerve during the pharyngeal phase of swallowing in infant animals simply implies longer contraction of the tongue muscles concerned.

In adult animals, the firing is brief and discrete, implying brief contraction of the tongue, linked with swallowing. It is possible that the asynchronous firing and longer contraction in the immature animals reflects difference in tongue movements as well as immaturity of reflex swallowing. So far, I have no evidence that squirt of a small amount of water directly into the pharynx of infant animals produces a suckle movement which might be indicated by this asynchronous firing of the hypoglossal nerve fibers, in contrast with similar elicitation of a swallow movement in the adult.

KENNY: In terms of our own work on the tongue protrusion reflex, it would be interesting to know if the tongue was either protruding or retruding progressively, in correlation with maturation of the reflex swallowing activity.

SUMI: I am also very much interested in knowing the changes of tongue movement associated with maturation of reflex swallowing. However, in my experiment on hypoglossal single fiber analysis there is no clue to protrusion or retrusion, since the tongue muscles are isolated from their innervating nerve fibers. To answer your question, it will be necessary to utilize other experimental models.

Chapter 11

INVESTIGATIONS OF TASTE FUNCTION AND SWALLOWING IN FETAL SHEEP

Robert M. Bradley and Charlotte M. Mistretta

In the *Third Symposium on Oral Sensation and Perception* two chapters describe morphological studies of developing gustatory receptors (5, 20). In these chapters it is noted that taste buds develop long before birth in some mammals. The presence of fetal taste buds suggests a number of questions: (1) Are the taste buds functional *in utero?* (2) Can a fetus perceive, or respond to, taste sensations *in utero*? (3) Does the fetus use its sense of taste to monitor its liquid environment?

To answer these questions we began to study the development of taste function in fetal sheep. The long gestation of the sheep (147 days) and the large size of the fetus (~ 1,000 g at 100 days) make it well-suited for developmental studies. Since no information was available on the development of sheep taste buds, we initially made a histological study to trace the time course of the development of gustatory receptors. The first section of this paper describes the morphology of fetal sheep taste buds. To study functional development of the taste system, electrophysiological recordings were made of activity in the chorda tympani nerve while stimulating the fetal tongue with chemicals. Neural responses recorded from the chorda tympani are described in the second section of this paper. To determine whether the fetus can perceive, or respond to, taste stimuli, we have begun to study fetal swallowing *in utero*. Preliminary data on fetal swallowing are presented in the third section of this paper.

MORPHOLOGICAL STUDIES OF FETAL TASTE BUDS

Tongues from 10 fetuses at various ages from 50 to 134 days of gestation were removed and fixed in formal saline. Tongues from two newborn lambs and two adult sheep were also fixed. Tissues were embedded in paraffin and sectioned at 10 μ. The sections were stained with hematoxylin and eosin or with a silver stain for nerves.[23]

As early as 50 days of gestation cell

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collections or presumptive taste buds are present in the epithelium overlying fungiform papillae (Fig. 11–1,A). These early taste buds are morphologically similar to those described in humans between 9.5 and 11.2 weeks gestation (^{5}fi^{10}). The dorsal tongue epithelium at this age is composed of only two cell types and the cells of the superficial layer cover the presumptive taste buds.

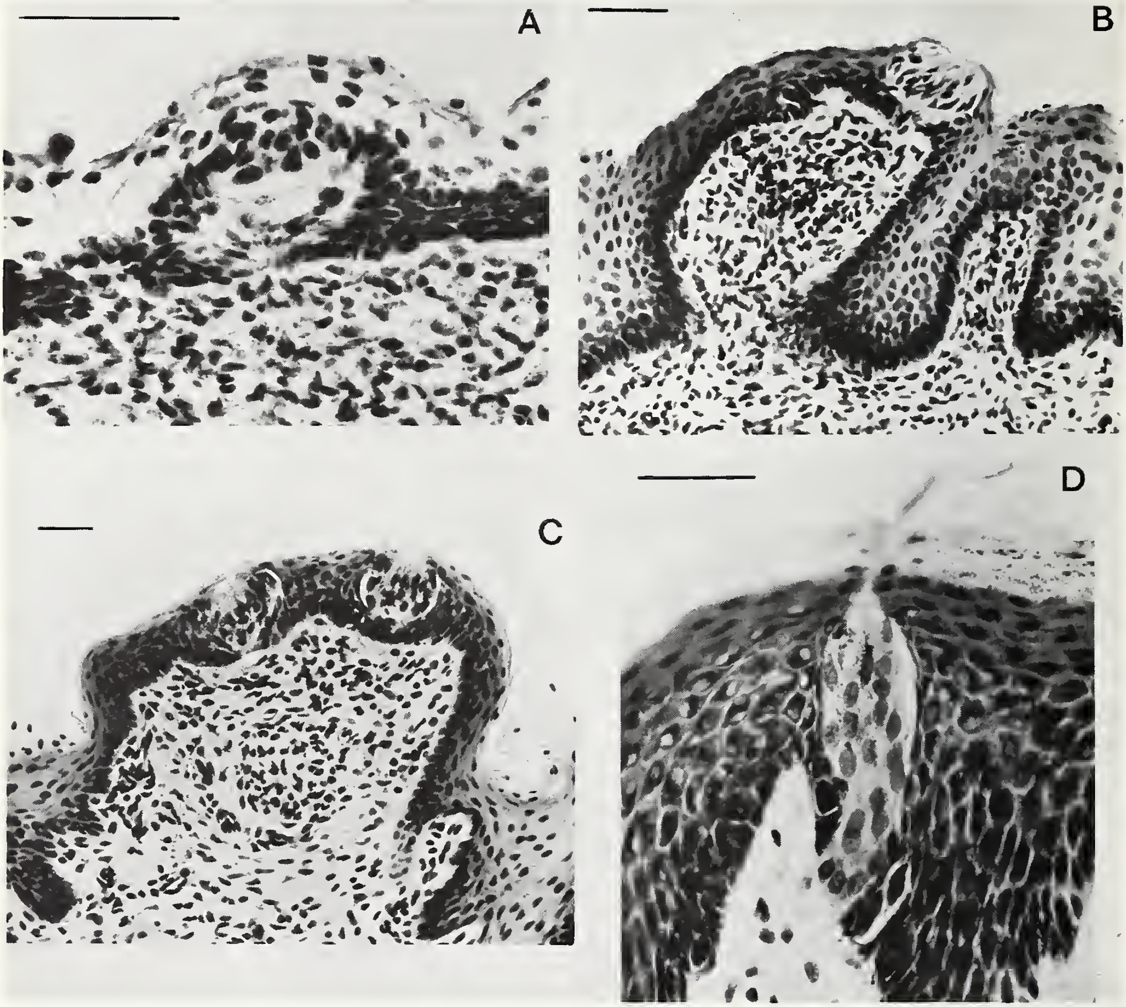

Figure 11–1. Photomicrographs of longitudinal sections through fungiform papillae and taste buds on the anterior third of the sheep tongue at various ages. The scale marked on each photograph represents 50 μ. A. Fungiform papilla and presumptive taste bud in a 50 day fetus. The disoriented cells of the early taste bud are covered by superficial cells of the tongue epithelium. H&E. B. Fungiform papilla and taste bud in a 100 day fetus. The papilla and taste bud are covered by superficial cells with flattened nuclei except for an area over the center of the taste bud. The first distinct signs of the taste pore are seen at this age. H&E. C. Fungiform papilla and two taste buds in a 134 day fetus. Cornified cells are present on the dorsal surface of the epithelium. A taste pit is present at the apex of each bud. H&E. D. Taste bud in an adult sheep tongue. Layers of cornified cells cover the fungiform papilla. The taste pit communicates with the oral cavity via a narrow channel which penetrates the cornified layers. H&E.

By 84 days the epithelium still consists of two cell types; however, the apices of the cells of the taste bud are no longer covered but are now exposed to the oral cavity. The taste bud cells have by this time assumed a perpendicular orientation to the basement membrane.

At about 100 days the apices of the taste bud cells are covered by cells with flattened nuclei and distinct staining characteristics (Fig. 11–1,B). Only a small area (~ 5 μ diameter) of the upper surface of the taste bud remains uncovered. It is, then, at this age that the first distinct signs of a pore are seen at the apex of the taste bud (see also 6, 8). By 134 days (Fig. 11–1,C) cornified cells are found on the dorsal surface of the tongue epithelium and a taste pit is obvious at the apex of each bud. In the adult sheep the depth of the cornified cell layers over fungiform papillae is approximately 10 μ (Fig. 11–1,D). The rather wide taste pit communicates with the oral cavity via a narrow channel piercing the cornified tissue.

The size of the fungiform papilla increases from ~ 60 μ diameter in the 50 day fetus to ~ 380 μ in the 134 day fetus. Although only one taste bud is seen per papilla between 50 and 100 days of gestation, after 100 days two taste buds are frequently found per fungiform papilla. In the adult sheep the fungiform papilla is about 800 μ in diameter and contains up to 8 taste buds. The mechanism through which the number of taste buds per fungiform papilla increases is not known.

Nerve fibers penetrate the connective tissue core of fungiform papillae and reach the basement membrane of the epithelium in 50 day fetuses (Fig. 11–2,A). In some tissue sections fibers

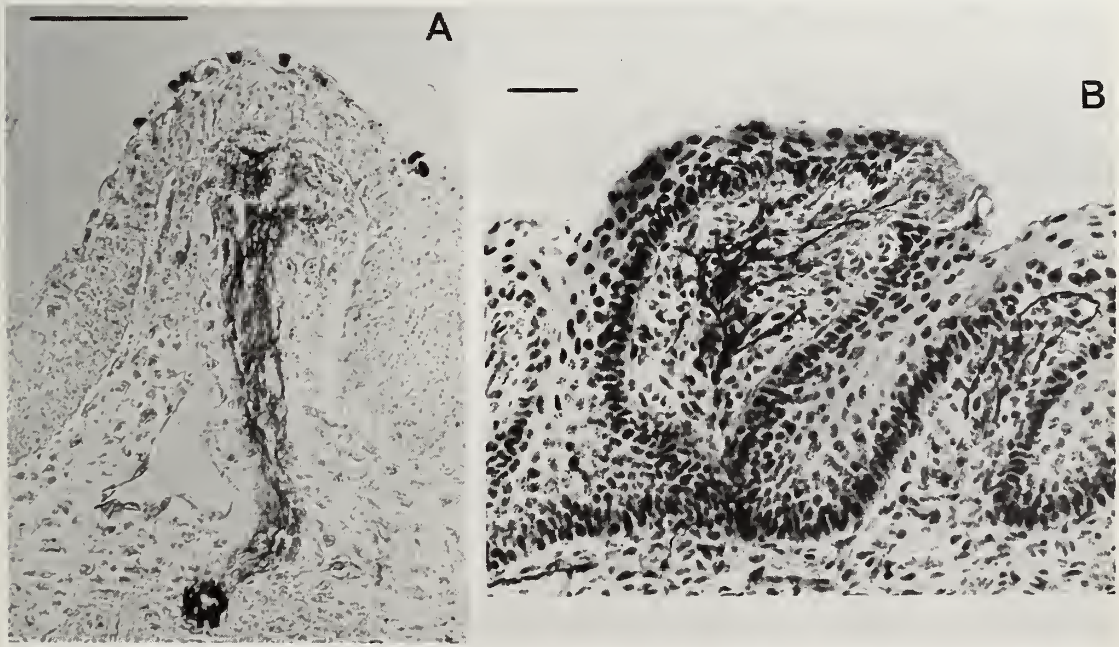

Figure 11–2. A. Fungiform papilla in a 50 day fetus. A bundle of nerve fibers penetrates the connective tissue core of the papilla. Sevier-Munger; scale = 50 μ. B. Fungiform papilla in a 100 day fetus. Nerve fibers are seen within the taste bud. Fibers course to regions of gustatory and non-gustatory epithelium. Sevier-Munger; scale = 50 μ.

penetrated the basement membrane.[8] By 100 days distinctly staining fibers were seen within the taste bud (Fig. 11–2,B). The fibers branch from the center of the papilla and course to regions of gustatory and non-gustatory epithelium.

FETAL CHORDA TYMPANI NERVE RESPONSES

From the light microscopic studies of the developing taste bud it was found that by about 100 days the fetal taste bud has a taste pore and a well developed nerve supply, and appears similar to the adult bud morphologically. Therefore, it might be expected that the fetal taste system would function from at least 100 days of gestation. To determine if the fetal taste receptors are functional, recordings were made of activity in the chorda tympani nerve while chemically stimulating the tongues of fetuses.

Methods

Thirty-five fetuses aged 96–137 days gestation were used. A detailed description of the surgical preparation and recording conditions has been published.[8] Briefly, both mother and fetus were anesthetized via an injection of sodium pentobarbital into the maternal jugular vein. The fetus was delivered onto a heated table and the umbilical circulation remained intact. Maternal and fetal blood PO_2, PCO_2 and pH were determined hourly to assess the condition of mother and fetus.

The fetal chorda tympani nerve was exposed by a lateral approach and dissected for either single or multi-fiber recordings. Using a pre-amplifier, oscilloscope and audio monitor, recordings were made of neural activity. Multifiber activity was integrated electronically,[3] using a time constant of 0.5 sec, and fed to a pen recorder. The magnitude of the integrated response was proportional to the level of neural activity at any given time. Single unit responses were recorded on magnetic tape for later analysis, Recordings of multifiber activity were also made from the chorda tympani nerves in five newborn lambs and six adult ewes. All chemicals used as stimuli were dissolved in distilled water and kept at room temperature during experiments. Stimuli were applied to the anterior third of the tongue using 20 ml plastic syringes. Stimuli were rinsed from the tongue with distilled water.

Results and Discussion

Neural responses were recorded from the chorda tympani nerve during chemical stimulation of the tongues of fetuses aged 96 to 137 days (Fig. 11–3). Stable

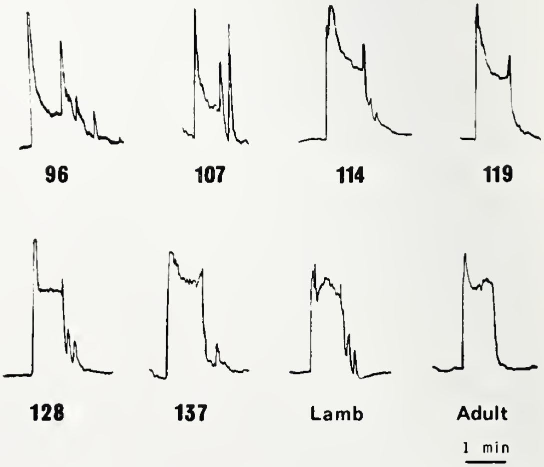

Figure 11–3. Integrated responses recorded from the chorda tympani nerve while stimulating the tongues of fetuses, a newborn lamb and an adult with 0.5 M NH_4Cl (exception: the 96 day fetal tongue was stimulated with 0.25 M NH_4Cl). Fetal gestational ages are noted beneath responses.

preparations lasting for periods of hours could only be maintained in fetuses aged 118 days and older. In fetuses of 100–110 days gestational age it was extremely difficult to record reproducible responses to repeated stimulation. In older fetuses, newborn lambs and adults it was found that 0.5 M NH_4Cl was an effective "standard" stimulus; i.e. lingual stimulation with 0.5 M NH_4Cl elicited a relatively high magnitude neural response and on repeated applications of this chemical, a reproducible neural response was obtained. To obtain reproducible responses on repeated stimulation in fetuses of about 100 days, however, it was necessary to use 0.1 or 0.25 M NH_4Cl. Even with lower concentrations of stimuli, the youngest preparations were difficult to hold beyond 15 or 20 minutes. We do not yet understand whether the inability to obtain stable responses is related to lack of cornified tissue on the tongue (which normally prevents rapid penetration of chemicals through the epithelium)[19] or to receptor or nerve fiber fatigue. Signal-to-noise ratios were usually low in fetuses aged 118 days and younger. The chorda tympani nerve was very fragile in younger fetuses and presumably, easily damaged.

Ten single or few fiber recordings were made in fetuses. All of these preparations responded with a relatively high frequency discharge to lingual stimulation with 0.5 M NH_4Cl (Figs. 11–4, 11–5 and 11–6). Sugars were not effective stimuli although glycerol, glycine and Na saccharin, which taste sweet to man, did elicit a response. One fiber responded to lingual stimulation with amniotic fluid collected during delivery of the fetus (Fig. 11–5), although other fibers tested with amniotic fluid did not respond.

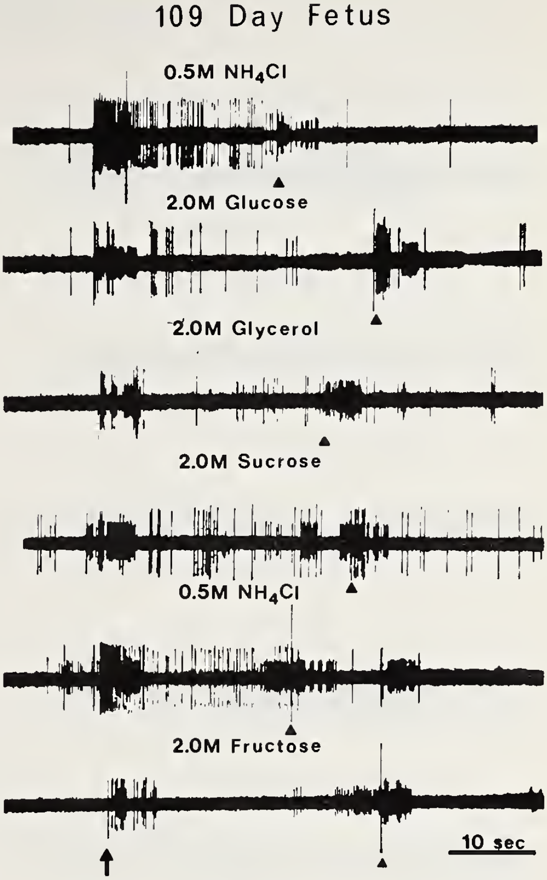

Figure 11–4. Neural impulses recorded from a few chorda tympani fibers in a 109 day fetus. Arrow at the bottom left of the column indicates time of stimulus application. Arrow heads in each trace indicate distilled water rinses. The record is continuous from top to bottom.

Generally, in fetuses, lambs and adults, salts and acids elicited neural responses of greater magnitude than chemicals which taste sweet or bitter to man (Fig. 11–7). This agrees with the data of Bell and Kitchell[4] on adult sheep chorda tympani responses. No fetal chorda responses could be recorded during lingual stimulation with 1.0–2.0 M fructose, glucose or sucrose. Responses to fructose and sucrose were, however, recorded in newborn lambs and adults (Fig. 11–7). Iggo and Leek[15] have sug-

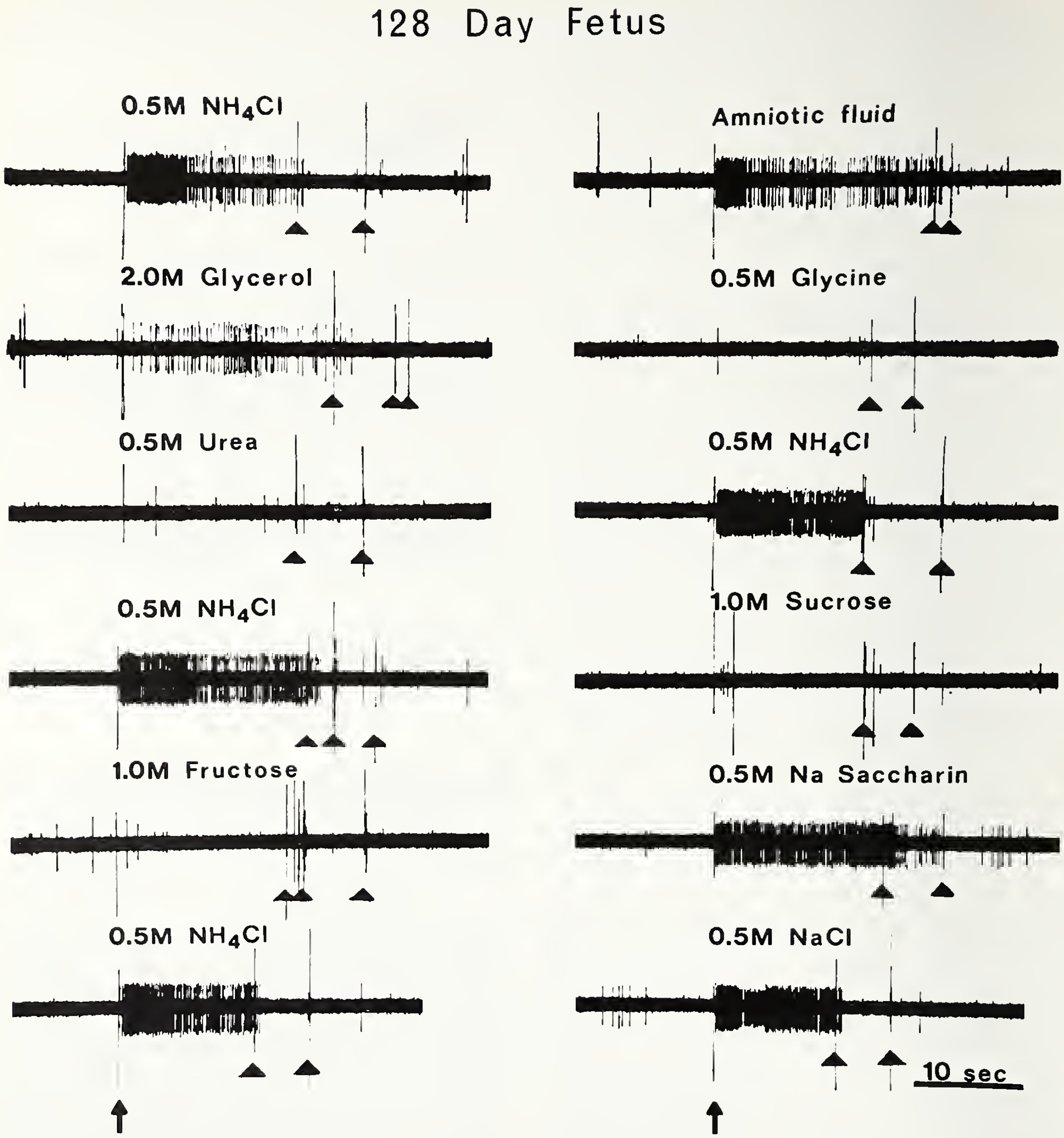

Figure 11–5. Neural impulses recorded from a single chorda tympani fiber in a 128 day fetus. Arrows at the bottom of each column denote time of stimulus application. Arrow heads in each trace indicate distilled water rinses. The record is continuous, and traces overlap, when read from top to bottom, left column, then right.

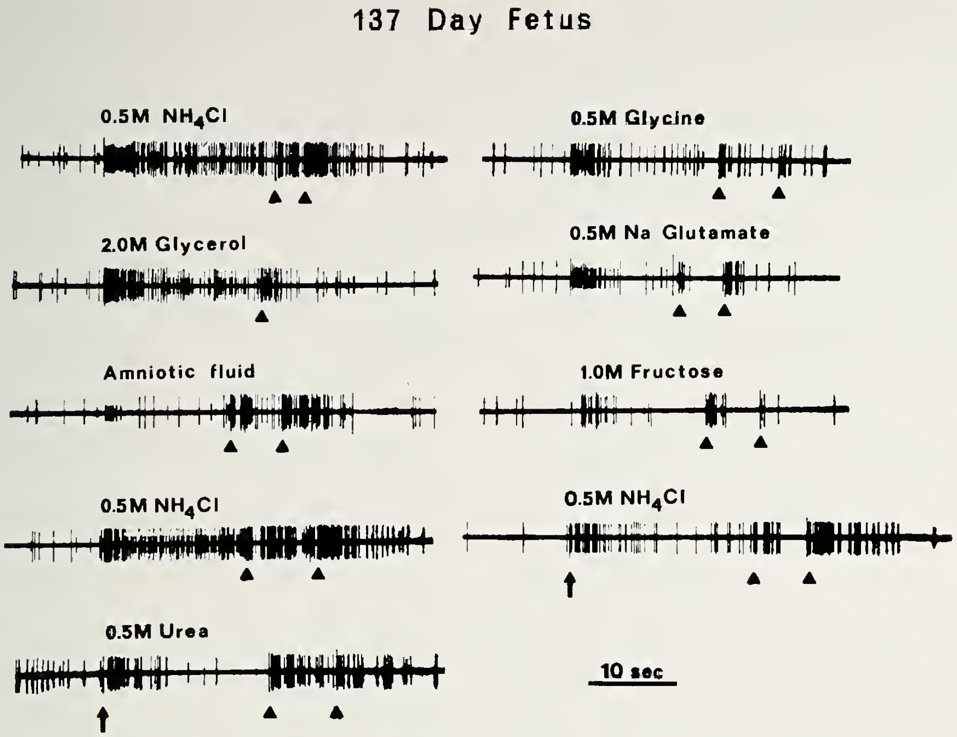

Figure 11–6. Neural impulses recorded from two chorda tympani fibers in a 137 day fetus. Arrows at the bottom of each column denote time of stimulus application. Arrow heads in each trace indicate distilled water rinses. The record is continuous, and traces overlap, when read from top to bottom, left column, then right.

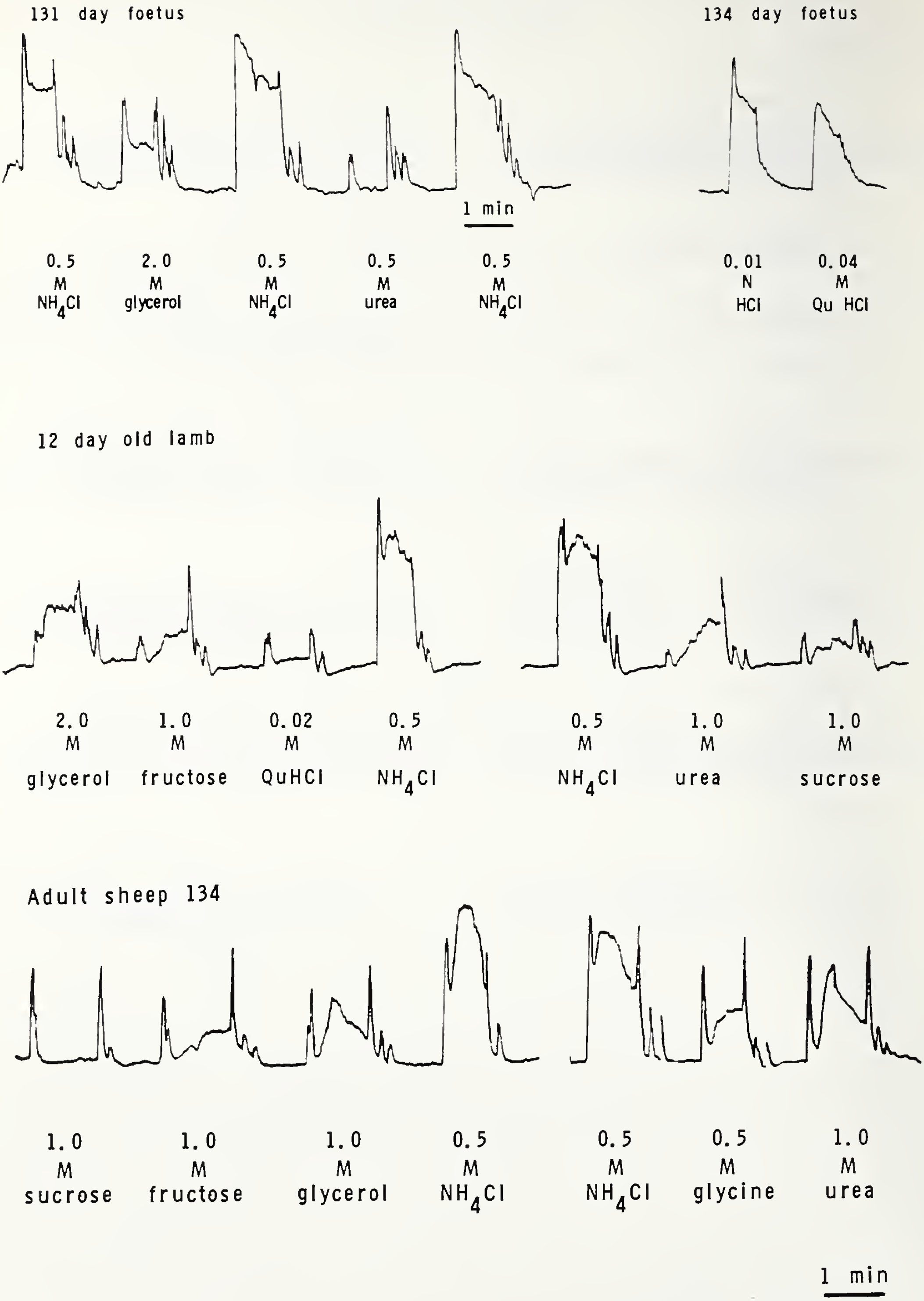

Figure 11–7. Integrated responses recorded from multi-fiber preparations during lingual stimulation with a variety of stimuli. In general, salts and acids elicited neural responses of greater magnitude than chemicals which taste sweet or bitter to man.

gested that the "sweet" fibers are of a very small diameter in sheep. Fibers of a small diameter would presumably be difficult to record from, especially in fetal preparations where signal-to-noise ratios were sometimes low.

When a series of 0.5 M monochloride salts was applied to the tongues of fetuses, the order of effective stimulation was $NH_4Cl > LiCl > NaCl = KCl$.[8] This order was also found in newborn lambs and in some adults. In other adults the order varied. When a concentration range from 0.1 to 0.75 M NH_4Cl was used to stimulate fetal, newborn and adult tongues, increasing concentrations elicited responses of increasing magnitude (Fig. 11–8).

The electrophysiological investigations have demonstrated that the peripheral gustatory system of the fetal sheep is functional over the last third of gestation. The fetal taste receptors seem comparable to those of the adult sheep in the range of chemicals to which they respond. It would be of great interest to record from the taste nerves in fetuses 100 days and younger, during the period when gustatory receptors are maturing morphologically. The fragility of the fetal nerves will limit the ages at which peripheral nerve recording techniques can be applied. Nonetheless, for detailed correlation between developing structure and function it will be necessary to record from the taste system in fetuses aged about 40–100 days.

The fact that neurophysiological responses can be obtained from fetal taste nerves while stimulating the tongue does not demonstrate that the fetus can actually taste or respond to chemical stimuli. We have begun to study fetal swallowing *in utero* and hope to use swallowing as an indicator of fetal behavioral taste responses.

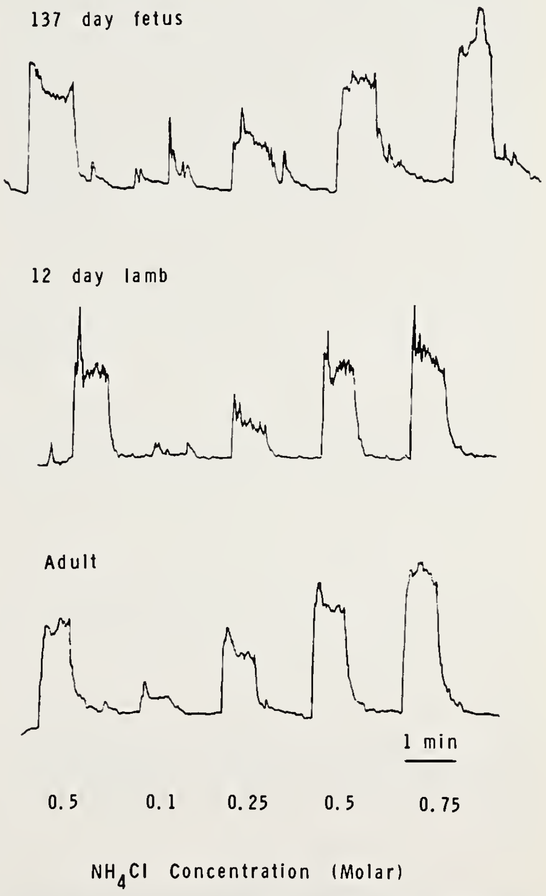

Figure 11–8. Integrated responses recorded from multi-fiber preparations during stimulation of the tongue with increasing concentrations of NH_4Cl. Increasing concentrations elicited neural responses of increasing magnitude.

SWALLOWING IN FETAL SHEEP

It is now well established that many mammalian species, including rats, guinea pigs, sheep and humans,[16,2,7,21] swallow *in utero*. The methods used to study swallowing usually involve injection of a tracer substance into the amniotic fluid. The tracer may be a radioopaque material, a dye or a radioisotope. Subsequent to injection, appearance of the tracer in the fetal gastrointestinal tract is appropriately measured, as is disappearance of the tracer from the amniotic fluid. These methods have yielded estimates of the volume of amniotic fluid swallowed by a fetus per day. They cannot, however, provide the exact volumes swallowed or indicate how frequently the fetus swallows.

Since we wished to use fetal swallowing as an index of taste preference behavior, we needed a more detailed knowledge of fetal swallowing activity. By implanting an electromagnetic flow transducer in the esophagus of the fetal sheep we have been able to continuously record swallowing. We hypothesized that if the amniotic fluid was flavored with a palatable chemical, the fetus would swallow increased volumes; if flavored to taste unpleasant, the fetus would either decrease or stop swallowing activity. Before modifying the taste of the amniotic fluid, it was important to know how frequently the fetus swallowed and how much it consumed. Baseline data on frequency of swallowing and volumes swallowed are reported here.

Methods

Data are presented for 10 fetuses aged 101 to 136 days at operation. The average duration of these preparations was 10 days. The basic surgical procedure has been described in detail by Dawes *et al.*[13] Under epidural anesthesia the pregnant ewe was prepared for surgery. Catheters were inserted in the ewe's carotid artery and jugular vein under local anesthesia. Nembutal was injected in the jugular vein for sedation (~ 7 mg/Kg). The uterus was exposed through a midline abdominal incision and the fetal head was located, pulled through the incision, and delivered Loss of amniotic fluid was minimized by clamping the uterine and fetal membranes to the skin of the fetal neck. A cannulated electromagnetic flow transducer[11] was inserted into the lumen of the fetal esophagus and the incision was closed (Fig. 11–9). Catheters were inserted into the fetal carotid artery and trachea. One amniotic fluid catheter, for delivery of chemical solutions, was either (a) sutured to the fetal head and snout, with the end over the nose; or (b) placed with the end in the fetal mouth, sutured externally to the cheek. A second catheter, for sampling amniotic fluid, was sutured to the back of the fetal neck. All catheters were externalized through two puncture incisions in the ewe's flank.

The ewe was placed in a metabolism cage after surgery. Fetal carotid and tracheal catheters were connected to pressure transducers and the respective pressures were continuously recorded. The output of the flowmeter was recorded continously, giving a record of fluid flow in the esophagus. A record of

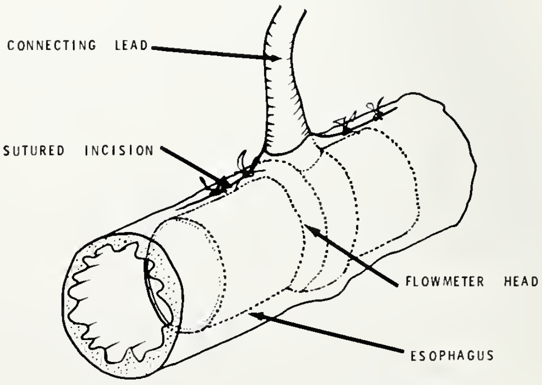

Figure 11–9. Diagram of cannulated electromagnetic flow transducer in the fetal esophagus, to illustrate placement technique.

integrated flow provided volume swallowed at any given time. Maternal and fetal blood samples were analyzed daily for PO_2, PCO_2 and pH.

Results

Swallowing results in a net flow of fluid down the esophagus, recorded as positive pen deflections (Fig. 11–10). The recordings obtained in chronic preparations were comparable to those obtained during acute experiments when an observer actually saw the fetus swallowing. Over the last third of gestation, the sheep fetus swallows large volumes of amniotic fluid in 2–7 "bouts" per day. A bout is a period of 1–9 minutes during which 20–200 ml of amniotic fluid are consumed. These bouts are usually accompanied by a rise in arterial pressure of 10–20 mm Hg (Fig. 11–11). Between the swallowing bouts the fetus frequently swallows smaller volumes (1–10 ml). The large volume swallowing episodes do not seem to occur at predictable intervals, but episodes are usually separated by at least one hour. In Figure 11–12 the hourly volumes swallowed by Fetus 170 over 10 consecutive days are plotted. No obvious "pattern" of swal-

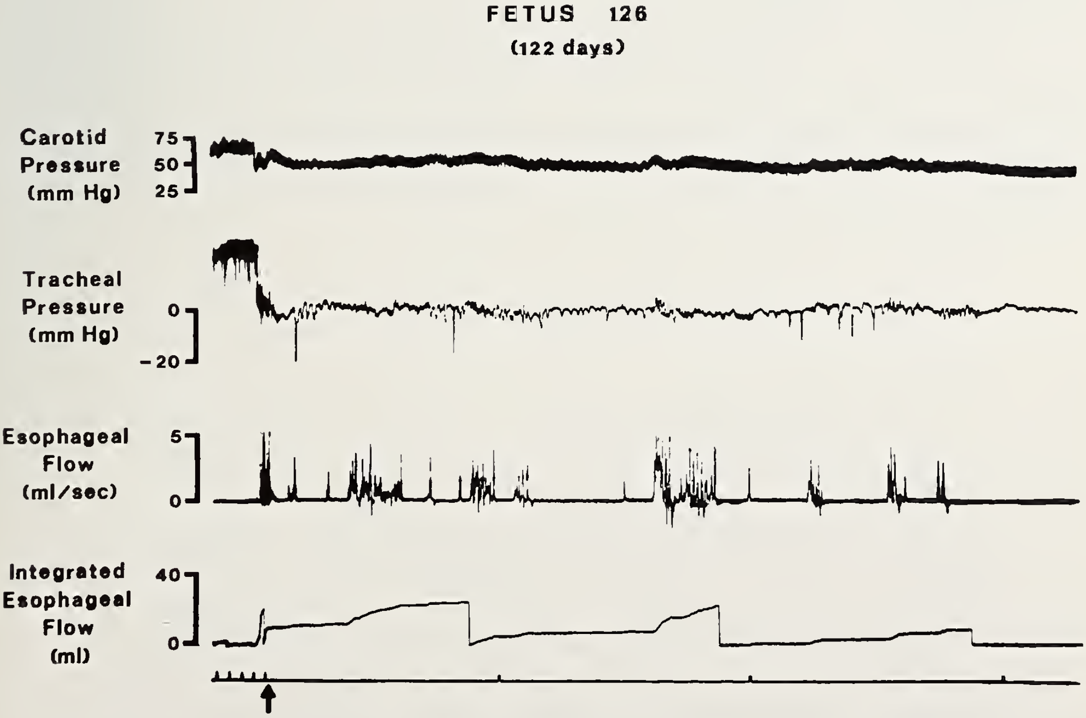

Figure 11–10. Record of swallowing activity from a 122 day fetus on postoperative day 4. At the point marked by an arrow the chart speed on the recorder was increased. Minutes are marked on the bottom line. The esophageal flow trace records the direct output from the electromagnetic flowmeter. The positive deflections result from swallowing. Integrated esophageal flow gives a measure of the volume swallowed. The integrator automatically resets to zero every 60 seconds. Tracheal and carotid pressures are also recorded. The large decreases in pressure recorded at the beginning of these two traces indicate that the ewe sat down from a standing position at the point of the pressure drop.

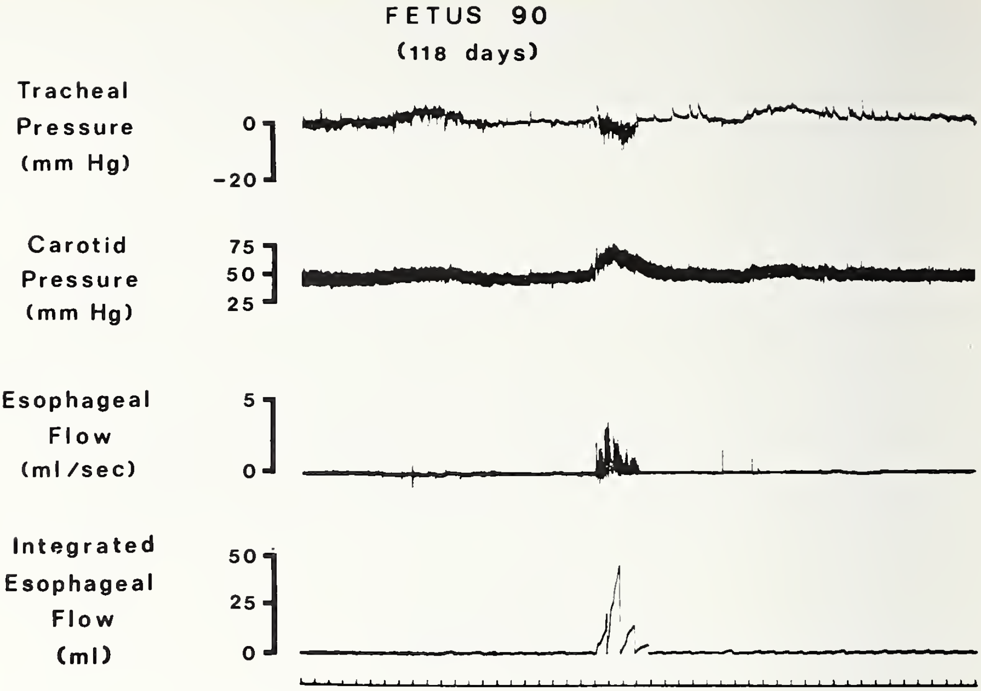

Figure 11–11. Record of swallowing activity from a 118 day fetus on postoperative day 3. Time is marked in minutes in the bottom line. The four minute swallowing bout seen in the middle of the recording was accompanied by a rise in arterial pressure.

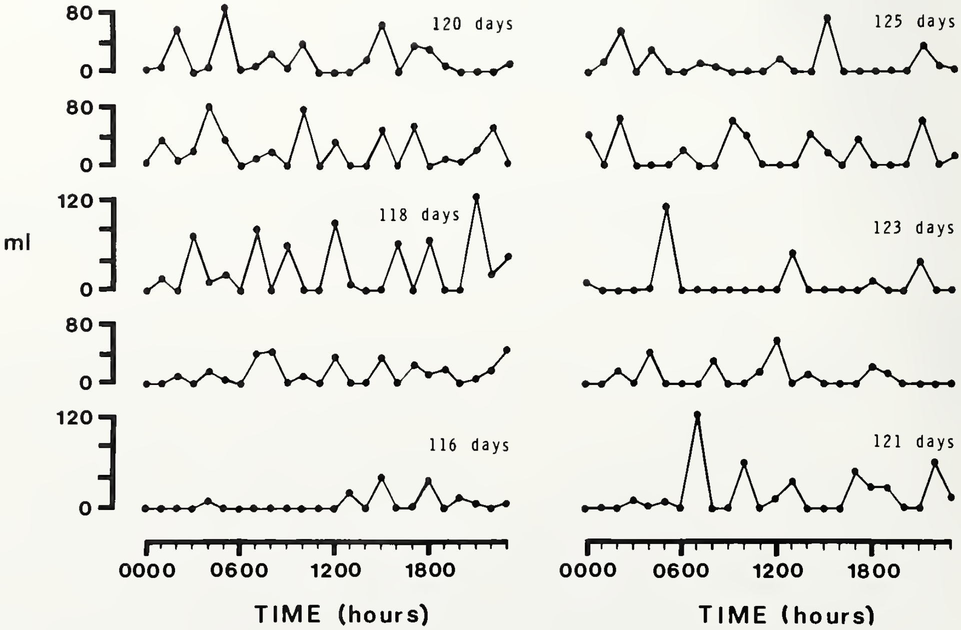

Figure 11–12. Hourly totals of volume swallowed by Fetus No. 170 for 10 consecutive days of the preparation. The fetus was operated at 115 days of gestation. No obvious pattern of swallowing was established.

lowing activity is seen. However, statistical analyses are necessary to resolve the question of whether the bouts occur at random.

Not only do the swallowing bouts seem to occur at unpredictable intervals, but also, the volume swallowed by a given fetus varies from day to day. Figure 11–13 illustrates daily volumes swallowed by Fetus 126 for the length of the preparation. For the first two postoperative days volumes swallowed were low, a peak volume was reached on the fourth day postoperatively and then, from the sixth through twelfth days a relatively constant volume was swallowed. Since chemicals were injected into the amniotic sac of Fetus 126 (see symbols in Figure 11–13) it is not possible to state that the increase in volumes swallowed from days 13–21 would have occurred in an undisturbed preparation. The increased swallowing and the decline in swallowing prior to labor may be related to glucose injections. However, we have determined that glucose levels in the amniotic fluid have returned to normal by ~ 20 hours after injection of the experimental concentrations. So a high glucose concentration was not maintained in the amniotic fluid over days.

The rise in volume swallowed per day to a peak on the third to fourth postoperative day was observed in six fetuses and is thought to be related to fetal recovery from operative procedures. Mellor and Slater[18] have reported that following fetal surgery the amniotic fluid does not return to a stable composition for about 3–5 days postoperatively. This would presumably influence swallowing activity.

Since the flow transducer cannula does not completely occlude the fetal esophagus, we are probably not recording total volumes swallowed. During

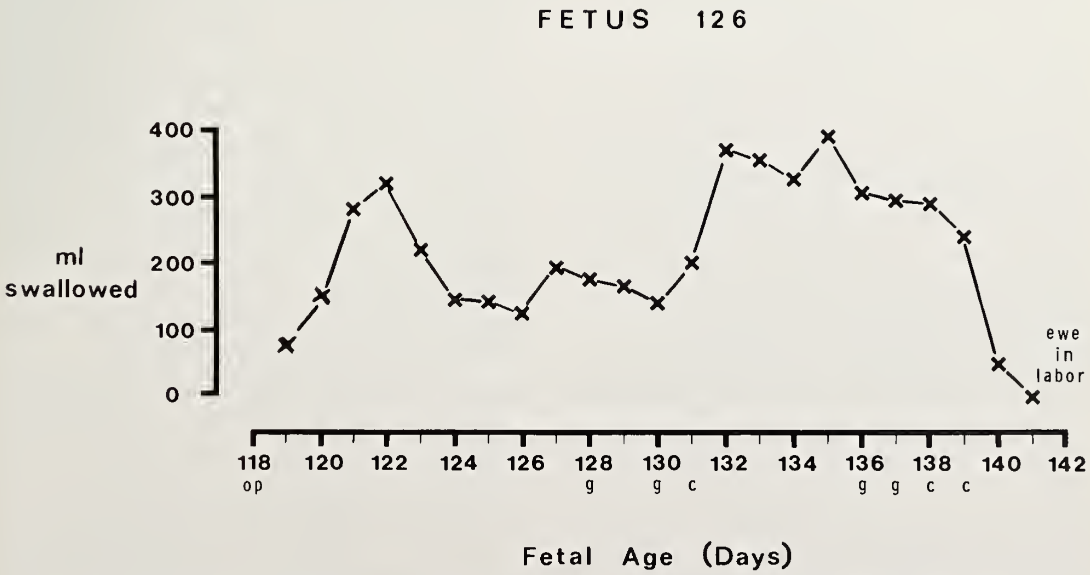

Figure 11–13. Daily volumes swallowed by Fetus No. 126 for the length of the preparation. The fetus was operated at 118 days of gestation. Letters under various gestational ages denote days on which chemicals were injected into the amniotic fluid; g = glucose, c = 0.154 M NaCL.

swallowing, some of the amniotic fluid may flow around the sides of the cannulated head instead of through the cannula. In eight fetuses from which swallowing data was collected for three days or more, average volumes swallowed ranged from 79 to 477 ml/day.[9] Wide variability in swallowing has been reported among human fetuses, also.[14,21] There are not enough data available to attempt any correlation between volume swallowed and size or age of fetus.

In a few preliminary experiments we have been able to artificially induce a swallowing bout by injecting 25 ml of 50% glucose into the amniotic fluid. However, in some fetuses attempts to induce swallowing by this means have failed. Control injections of saline and mechanical mixing of the amniotic fluid have never induced a swallowing bout. Many more experiments will be needed before we can determine if we have modified fetal swallowing by injection of chemicals into the amniotic fluid.

Discussion

The volume of amniotic fluid in the pregnant ewe at 112 days gestation is about 750 ml; by 140 days the volume has increased to about 1500 ml.[17] The daily volumes swallowed by the fetus represent a large proportion of the total amniotic fluid. It is interesting to speculate on the factors which may induce the fetus to swallow large volumes of amniotic fluid four or five times a day. It may be that the fetus becomes active during certain periods of the day and swallows at these times, simply because it is in an active state. Or perhaps fetal swallowing is in some way related to developing hunger or thirst mechanisms. On the other hand, it is possible that the fetus swallows as a result of stimulation of oral receptors.

From about 115 days of gestation, copious production of oral mucoid secretions has been observed in fetal sheep in acute preparations (personal observations). Also, outpourings of tracheal fluid into the oro-pharynx take place *in utero*.[12] It is not known when the salivary glands of the fetal sheep begin to function, but presumably, some saliva is produced prenatally. All of these various secretions provide potential sources of stimuli for oral receptors.

Fetal urine, by altering the composition and taste of the amniotic fluid, may stimulate the taste buds and thereby induce swallowing episodes. The volume of urine produced by the fetal sheep aged 120 days to term ranges from 173 to 677 ml/day,[1,22] similar to the range of volume of amniotic fluid swallowed. If the fetus produces relatively large volumes of urine at discrete times of the day, then the fetus may be able to detect the urine by means of its taste system. The presence of urine could thereby initiate swallowing.

As yet it is not possible to demonstrate any inter-relations between fetal swallowing activity and the fetal gustatory sense. There are, however, many chemicals in the amniotic fluid, including NaC1, KC1, fructose, glucose, urea, creatinine, amino acids, which provide potential stimuli to the taste system. Since the concentrations of these chemicals alter during gestation,[18] the taste of the amniotic fluid is probably changing. Whether swallowing is induced by stimulation of the taste receptors or not, certainly the taste buds are exposed during swallowing bouts to chemical stimulation. The act of swallowing amniotic fluid may be the organism's first feeding and tasting experience, and it may play a role in the development of mammalian taste preferences.

SUMMARY

Taste buds have begun to form on the tongue of the fetal sheep by 50 days of gestation (term = 147 days). The taste receptors at 100 days possess a taste pore and a well developed nerve supply. Electrophysiological experiments have demonstrated that the peripheral gustatory system is functional from at least 100 days gestational age. Since the taste system is functional, it may be that the fetus can actually perceive, or respond to, the taste of its amniotic fluid. Taste receptors in the fetal oral cavity are particularly accessible to chemical stimulation during the episodes of fetal swallowing. The fetus swallows large volumes of amniotic fluid during two to seven bouts per day. It is possible that the swallowing episodes are related to changing tastes of the amniotic fluid.

It is evident from this study that certain oral structures and functions are developed well before birth in the mammal. The extent to which the fetus may utilize its oral cavity to monitor its liquid environment remains to be elucidated.

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DISCUSSION

J.W. BROWN: This is rather interesting because we have photographic documentation that the human fetus swallows in response to perioral stimulation at 12.5 weeks of menstrual age and possibly earlier (10 weeks, but no photographs available). Also there is evidence that a gag reflex can be elicited about 18.5 weeks of menstrual age.

The matter of the influence of prenatal activity on the further development of neural pathways is a controversial subject about which Dr. T. Humphrey and I had numerous discussions. Several of her papers (*e.g.*[1]) presented evidence in favor of such a relationship. I am quite convinced that activity *in utero* can enhance the development of neural arcs by one or more possible mechanisms, *e.g.*, activity may favor the establishment of synaptic connections between the appropriate neurons forming the arcs or activity may result in the establishment of a "memory trace" by changes in RNA in the neurons participating in the response in a similar manner as has been suggested for adult learning processes. Moreover, a number of prenatal reflexes are repeated postnatally[2] indicating postnatal utilization of neural arcs established prenatally.

MALLER: It seems to me, looking at your electrophysiological data, that the concentrations of tastants you used are very high before you observe changes in electrical activity.

BRADLEY: The concentrations of gustatory stimuli used were indeed high, but similar concentrations were used on fetal, newborn and adult sheep. High concentrations were used by Bell and Kitchell[1] when recording from the chorda tympani in adult sheep. It seems that the fetus is like the adult in that fairly high concentrations have to be used to produce an adequate response from the whole chorda tympani nerve. Perhaps lower concentrations may be required if the receptors supplied by the glossopharyngeal nerve were stimulated. Bell and Kitchell were able to record relatively larger responses to acid and quinine and to solutions that taste sweet to man from the glossopharyngeal nerve.

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MALLER: This is also suggested by Bernard's[2] work on electrophysiological recordings of peripheral nerves of the calf in response to chemical and mechanical stimulation of the tongue.

Have you any information about the taste preferences (behavioral preferences) of newborn lambs? These data would seem to be a more useful guide to altering the palatability of amniotic fluid. You seem to be relying very heavily on your electrophysiological data for information about what are adequate taste stimuli for sheep. As you are aware there is some discordance between electrophysiological measures and behavioral indices of taste, particularly in regard to "sweet" taste of sugars.[3,4]

BRADLEY: We are not using the electrophysiological data to choose stimuli for behavioral studies, but to determine: (a) if the peripheral taste system is functional, and (b) if the electrophysiological response characteristics change as the system matures. There is some data on taste preferences in adult sheep[1,2,3,4,5] which we have used in selecting stimuli for palatability studies. We have also done some preliminary testing of newborn lambs ourselves.

WEIFFENBACH: I am particularly interested in the burst activity in your single fiber recordings. As I understand, this bursting phenomena is different from the change in base rate of firing occurring under sustained stimulation with a sapid solution. The bursting is a transient phenomenon that appears to accompany the change in stimulation from sapid solution to water rinse and vice versa. The possibility exists, of course that these bursts are related quite closely to the stimulus, the classical "on-off response", with a burst following the beginning of the sapid solution stimulation (an "on response") and a second (an "off response") following conclusion of this stimulus. In our concern with the sapid solution as the stimulus, we readily accept the idea that there might be a neural response to the onset and to the termination of that stimulus. It is also easy to believe that there might be a response to a change in stimulation without regard to whether this change is an increment or decrement in stimulation of the taste sensitive elements. However, I wonder if these are the most fruitful ways to look at the bursting phenomena. Is it possible that a second non-taste modality of lingual sensitivity is being stimulated at the transition from stimulus to rinse and back again? Is there a common physical or mechanical disturbance at the shift point that might excite touch or mechano-receptors? Would you get bursting if you shifted from a water "stimulus" to a water rinse?

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MISTRETTA: First, I would like to make it clear that we have not made a detailed study of responses from single chorda tympani fibers in fetal sheep. Those few from which we have recorded seem similar in response characteristics to chorda fibers in adults of various species. That is, chorda tympani fibers respond to *chemical stimulation* of the tongue with a variety of response patterns.[1] The patterns include: (1) *phasic tonic response*, characterized by a high frequency response at the onset of stimulation which decreases to a maintained, average response frequency; (2) *rhythmic bursting*, characterized by brief bursts of neural activity with longer, silent periods in between bursts; (3) *gradual increase in response frequency*, characterized by a quiet period after application of the stimulus and then a gradual build-up of response frequency; (4) *"on" response*, characterized by a very high frequency burst of impulses on stimulus application; a burst may or may not occur on rinsing the stimulus from the tongue; (5) *"off" response*, characterized by a very low response frequency during stimulus application followed by a high frequency discharge after rinsing; (6) *depression of spontaneous activity* during the period of stimulation.

All of these patterns represent taste, not tactile, responses, and I feel that each of these patterns may convey different kinds of information to the central nervous system. There has been too much emphasis in the taste literature on the typical phasic-tonic response and other temporal patterns have been largely ignored. Since the discharge of impulses by taste afferents is typically irregular, the temporal characteristics of the spike train may be of significance in the process of coding taste qualities.

When recording from multi-fiber bundles of chorda tympani fibers, responses will be recorded to the chemical, tactile and temperature properties of the stimulus. One way of avoiding tactile stimulation is to use a flow chamber which eliminates the tactile component of stimulus presentation.[1] Responses will then only be recorded from taste afferents, which are sensitive to chemical and temperature stimulation.

KAWAMURA: When we discuss swallowing behavior we must always consider the volume of swallowing. In these fetal sheep, what was the volume of salivation at rest? And what was the volume of gustatory reflex salivation?

MISTRETTA: We have not measured salivary flow in fetal sheep and are not aware of any studies on the development of salivary gland function in fetal sheep. The salivary glands have begun to differentiate in the 28 day sheep embryo,[1] however, and they are probably functional prenatally. While doing electrophysiological experiments, we have noted a copious mucoid secretion from the oral cavity of fetuses aged about 120 days and older. We have not localized the origin of these secretions. I would like to point out that we are studying a fluidfilled system *in utero*. However, we are open to the possibility that oral secretions and gushes of tracheal fluid may stimulate oral receptors and induce swallowing.

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BRADLEY: In adult sheep, a continuous, spontaneous secretion of saliva is produced by the parotid glands.[2,3] I wish we knew when these and the other salivary glands begin to secrete.

J.W. BROWN: In view of the early appearance of the gag reflex in the human fetus, have you any evidence of regurgitation or back flow in the flowmeter in the esophagus? I wonder if you were to make the fluid taste very unpleasant, *e.g.*, very bitter—an extreme level of a tastant, whether or not a reverse flow would occur?

BRADLEY: In regard to regurgitation, we have no evidence of it in fetal sheep. You may think that the fetal sheep in particular would regurgitate, in preparation for later rumination. However, neither the fetus nor the newborn lamb is a ruminant. In suckling ruminants, liquids are swallowed and pass directly to the abomasum or secretory portion of the stomach.[1] In the young ruminant both the reticulum and the rumen are small and undeveloped. The esophageal groove is responsible for the bypass of reticulum and rumen when liquids are swallowed.

KENNY: My question is related to your single fibre studies. I was wondering in fact if the ammonium chloride was stimulating pain receptors.

MISTRETTA: We chose 0.5 M NH_4Cl as our standard stimulus, that is, the stimulus which we used to test for reproducibility in the neural preparations. This stimulus was chosen because it elicited a relatively large magnitude neural response. On repeatedly stimulating the fetal, newborn and adult tongue with 0.5 M NH_4Cl, reproducible neural responses were recorded. In very young fetuses with no cornified epithelium on the tongue dorsum, it was necessary to use 0.1 M or 0.25 M NH_4Cl as the standard stimulus. On repeated stimulation with 0.5 M NH_4Cl at about 100 days of gestation, decreasing neural responses were obtained.

I see no reason to think that we were stimulating pain receptors. The neural responses which we recorded are typical taste responses, and I do not think we were recording from fibers of particularly small diameter. A rat will drink 0.1 M NH_4Cl;[2] it would be necessary to go to concentrations much higher than 0.5 M to elicit pain. I am not aware of any reports on neural responses from pain fibers in the chorda tympani.

J.W. BROWN: Most of the common reference texts do not mention pain fibers

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in the chorda tympani. There is, however, at least one, somewhat obscure, account which indicates that such fibers may be present. Costen, Clare and Bishop[1] reported a case where following a radical mastoidectomy the chorda tympani was exposed. Both mechanical and electrical stimulation of the exposed nerve resulted in pain sensation over the anterior two-thirds of the tongue. Later, section of the nerve resulted in loss of both pain sensation and taste over the anterior two-thirds of the tongue with preservation of tactile sensibility. They also made fiber size counts of the chorda tympani nerves from several human autopsy cases and from several macaques. Their results indicate the presence of somaesthetic sensory fibers of a size ascribed to pain fibers in addition to fibers presumed to mediate taste impulses.

With regard to taste, I wonder to what degree the responses to taste are learned postnatally? One might expect the finer degrees of discrimination of tastants to be the result of cortical associations developed after birth. Certainly a developing child exhibits changes, *e.g.*, a very young child may like beer—a year later he may dislike it. If there is a response to tastants prenatally (Steiner's later paper indicates this may be) then it might take place at a subcortical or brain stem level and if so it might be a less graded or discrete type of recognition, *i.e.*, one may need to use extremes of tastants very sweet or very bitter (approaching a noxious stimulus)—in order to get a change in the swallowing responses of fetal sheep.

BRADLEY: With respect to your question as to what degree responses to taste are learned postnatally, I would like to mention the existence of "critical periods" in development. For example, Hubel and Wiesel[1,2] have shown that in the newborn kitten visual stimulation is necessary for the formation of the neural connections associated with a normal visual system. Perhaps the amniotic fluid provides the fetus with an intrauterine taste stimulation necessary for the formation of the gustatory system. We hope to investigate this question.

MISTRETTA: I would like to add that attempts have been made to establish novel taste preferences in newborn animals through prolonged exposure of the newborn to a taste stimulus.[3,4] In guinea pigs a preference for a bitter solution was conditioned and in rats a preference for citral was conditioned. In both instances, though, the altered preferences were short-lived and the experimental newborns reverted to "control" behavior. In vertebrate classes other than mammals[5,6] experiments have demonstrated the importance of early experience on feeding preferences. Unfortunately, these studies do not trace subjects to adulthood, and so it is not known how long the effects of early experience last.

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STEINER: I would like to ask whether the bursts of the swallowing activity in the fetus correspond with noises coming from the environment, or with incidental touch stimuli. I am sure that you are aware of the possibility that noises originating from the external environment can induce general restlessness and motor activity in the fetus *in utero*. Such general motor activity can include also swallowing movements.

MISTRETTA: We are aware that external stimuli can elicit fetal responses *in utero*. Human fetal evoked responses have been recorded in response to acoustic stimulation[1,2] and heart rate changes have been recorded in response to sound and vibration.[3] The sheep used in our experiments were housed with one to two other sheep, and a number of investigators entered the sheep room at various times of the day and evening. So the ewes may have been frequently "disturbed" and exposed to acoustic stimuli, but we have no record of when humans did enter the sheep quarters. Certainly, between the hours of 2 a.m. and 6 a.m. the sheep were usually undisturbed; we find no evidence that the fetuses swallowed either more or less during this period.

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Chapter 12

NEUROPHYSIOLOGICAL MECHANISMS RELATED TO REFLEX CONTROL OF TONGUE MOVEMENTS

YOJIRO KAWAMURA AND TOSHIFUMI MORIMOTO

The tongue always exhibits highly organized, skillful movements for sucking, swallowing, chewing, yawning, crying and so on. Without well organized tongue movements, the infants would be seriously impaired in their milk sucking and would be unable to fulfill their normal healthy development.

The mouth is the only organ in the body in which we find a movable cavity containing such a complex movable organ as the tongue. We don't usually bite the tongue during routine movements of the mouth, and an intricate coordination can be recognized between the tongue and mandibular muscles during chewing, speaking, swallowing and in other oral performances. In addition, the tongue itself is capable of changing in shape and form purposefully in the process of chewing and speaking, and the tongue also can produce rapid movements in different dimensions. The well organized motor control of the tongue is attributed to specialized arrangement of the lingual muscles and to neuromuscular reflex mechanisms, although there are still many unexplained problems in mechanisms of the tongue reflexes.

The tongue is made up of two different kinds of muscle groups, that is, the extrinsic and intrinsic muscles.

The extrinsic tongue muscles attach to the skeleton (cranium or mandible) at one end and the other end of the muscle terminates within the tongue itself. On the other hand, the intrinsic tongue muscles begin and end within the tongue and they have no attachment to skeletal structures. When we discuss neurogenesis of well organized movements, at least, mechanisms of coordination of tongue and mandibular movements and of autonomous control of the tongue muscle itself must be considered.

Schoen[23] first recognized that contraction of the tongue was induced by passive depression of the mandible. Blom[2] also confirmed this phenomenon by recording the electromyogram (EMG) of the tongue muscles. On the other hand, Szentágothai[24] reported a morphological degeneration experiment in which the collaterals of the mesencephalic tract neurons could be traced to the caudal part of the ipsilateral hypoglossal nucleus, and he suggested these few collaterals could be considered as direct inhibitory fibers to some neurons in the hypoglossal nucleus. Following this assumption about the possible neural connection between the trigeminal mesencephalic nucleus and the hypoglossal nucleus, the proprioceptive impulses from the masseter muscles have been appropriately considered to contribute a significant role in the coordinated action of the tongue muscles during mastication[12,22] in electrophysiological experiments in cats. Mizuno and Sauerland[17] also demonstrated that proprioceptive muscle afferents from the masseter muscle project directly to the ipsilateral upper cervical ventral column.

However, EMG activity of the tongue muscles induced by mandibular opening was not eliminated even after bilateral section of the masseter nerve. In addition, direct electrical stimulation of the masseter nerve induced only the IPSPs of the hypoglossal motoneurons and inhibited spontaneous discharges of these neurons. This inhibitory effect strongly implies excitation of a high threshold afferent fiber in the masseter nerve.[20] For these reasons, the effects of afferent impuses from the masseter muscle upon the hypoglossal motoneurons may be attributed to sensory mechanisms which act via interneurons in the hypoglossal nucleus. These are mechanisms other than the spindle afferents from the masseter muscle.

Thus, up to the present time, the role of afferent impulses from the muscle spindles of the masseter muscle for control of the tongue muscle activities have been well considered and emphasized as background mechanisms for coordination between the tongue and mandibular movements. However, afferent impulses from other mandibular muscles must be also considered in these mechanisms.

In addition to the aforementioned reflex mechanisms for coordination between tongue and jaw movements, myotatic reflexes of the tongue itself also may play a very important role in regulation of motility of the tongue.

In humans and monkeys, muscle spindles have been found in the tongue[3,4,9] and stretch receptor discharges in the hypoglossal nerve have also been reported by Bowman and Combs.[5]

According to Cooper,[9] muscle spindles in the intrinsic muscles of the human tongue are few in number in the tip, in contrast with the numerous tactile endings in the tongue tip mucosa. Muscle spindles are richly distributed in the most flexible part of the human tongue: the region proximal to the tip. And the spindles are distributed most densely in the superior longitudinal and transverse muscles. Spindles are also found in the posterior part of the inferior longitudinal muscles and in the muscles near the lateral border which predominately participate in the fine lateral movements of the tongue. In the vertical tongue muscles, spindles are distributed fairly far forward in the tongue, where they are active in any of the tongue movements.

On the other hand, there is still some controversy concerning the existence of muscle spindles in the tongue muscle in subprimate mammals that could provide proprioceptive information. Some investigators have reported the presence of muscle spindles in the subprimate mammalian intrinsic tongue muscles, but some have reported absence of muscle spindles. The presence of muscle spindles in the tongue muscle was reported by Langworthy[14,15] in cat and by Tarkin[25] in rabbit. But no muscle spindles were found in the intrinsic tongue muscles of rabbit by Boyd[6,7] or Carleton,[8] of rat and rabbit by Weddel et al.,[26] or of pig, dog and cat by Law[16] However, other reports have shown a few atypical endings situated in the genioglossal muscle, and presumably indicated the possible existence of some other type of receptors for proprioception in the tongue muscle.[7,11,26]

Furthermore, in the cat, the possibility of existence of hypoglossal sensory fibers from atypical proprioceptors which subserve kinesthetic information from the tongue is strongly suggested by Morimoto and Kawamura[18] and by Zapata and Torrealba[27] on the basis of neurophysiological experiments, although several authors have been unable to record impulses from the hypoglossal nerve of the cat when they stretched the tongue.[1,2,10,21]

Impulses from the lingual nerve which are thought to be proprioceptive in origin have also been reported by Barron[1] and by Kawamura et al.[13] in the cat. And Porter[21] reported the existence of mechanorecptive afferent fibers in the lingual nerve. Kawamura et al[13] also reported mechanoreceptive afferent fibers in the glossopharyngeal nerve. In spite of absence of typical muscle spindles in the tongue muscle of subprimates, the tongue always shows intricate coordinated movements, and there must be some delicate autonomous neural mechanisms in well coordinated tongue movements of the subprimate other than the muscle spindle mechanisms.

Kawamura et al.[13] also recognized that a few fibers of the tongue muscles of a cat showed spontaneous EMG discharges and, among them, some increased their discharge frequency in response to stretch of the tongue. This response was not affected by anesthesia of the tongue surface but was abolished after section of the lingual and glossopharyngeal nerves and the lateral branch of the hypoglossal nerve. Therefore, the stretch responses of the tongue may possibly be elicited by excitation of deep mechanoreceptors in the tongue.

As mentioned above, there are still many unsolved problems concerning neuromuscular reflex mechanisms of tongue movements. In the present paper, some new concepts about neuromechanisms controlling coordination of tongue and jaw muscle are discussed upon the basis of experimental results in cats.

METHODS

1. *Interrelations between tongue and mandibular movements.*

Adult cats weighing 2.0–3.5 kg were used. They were anesthetized with hexobarbital sodium (40 mg/kg, i.p.) and, after precollicular decerebration and decerebellation, the animal was fixed in a supine position. Summated (EMG) activities of the tongue muscles were recorded by an electrical pulse integrator. EMG was recorded from implant electrodes consisting of a fine insulated copper wire (250 μ in diameter) bared for 1 mm. The wire was inserted into the middle of the muscle, with the bared part in some muscle bundles. In addition, a fine concentric needle electrode was used to record activities of neuromuscular units. EMG's were recorded from the styloglossus muscle, the anterior belly of genioglossus muscle and the hyoglossus muscle.

For electrical stimulation of the medial pterygoid nerve, the ipsilateral masseter muscle was stripped from its attachment to the mandible, and the zygomatic bone, the mandibular ramus, the condyle and coronoid process were removed. After these surgical procedures, the medial pterygoid nerve was located in the medial pterygoid muscle and exposed under observation with a stereoscopic microscope.

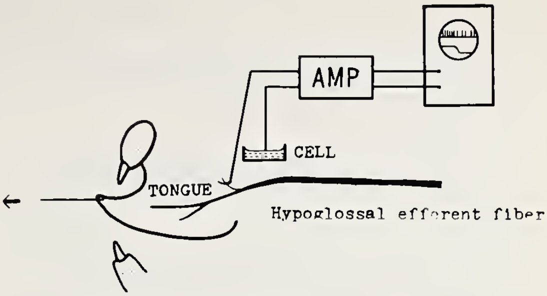

Figure 12–1. Schematic illustration of recording of responses from nerve fibers in the medial branch of the hypoglossal nerve during forward stretch of the tongue.

The stimulating electrode was a bipolar sleeve electrode made of two silver rings 7 mm apart, embedded into a polyethylene tube. The electrodes were covered with vaseline for insulation and fixed to the surrounding tissues. A single square pulse (duration of 0.01–0.1 msec) or repetitive stimulation was applied.

Response of a functional single unit of the hypoglossal efferent fibers induced by stimulation of the medial pterygoid nerve was also recorded. For this purpose, the central connection of the hypoglossal nerve was cut, and a peripheral end of the nerve was carefully dissected under observation through a stereoscopic microscope to obtain a functional single unit of the nerve. A monopolar silver wire electrode (100 μ in diameter) was used for recording of nerve discharges and a round silver plate 7 mm in diameter was fixed to the surrounding tissues as an indifferent electrode. The mouth was passively opened by a surgical thread attached to a lower canine and passed through a pulley system to a suspended weight.

2. *Autonomous control of tongue muscle activities.*

Decerebrate cats were used, and the experiment was performed under hexobarbital sodium anesthesia (40 mg/kg, i.p.). After the animal was fixed in a supine position, the hypoglossal nerve was exposed by a ventral approach. There are two branches in the hypoglossal nerve in the cat, that is the medial branch and lateral branch. However, Morimoto and Kawamura[18] reported that in the cat the afferent nerve fibers in the hypoglossal nerve which respond to foward stretch of the tongue are mostly in the lateral branch, which innervates the tongue retractive muscles; there were no afferent components in the medial branch. The lateral branch was kept intact in these experiments to retain these afferent components. A fine fiber bundle was separated from the whole medial branch, the central connection of the fiber bundle was cut, and a response induced by forward stretch of the tongue was recorded in this bundle transmitting efferent impulses from the hypoglossal nucleus. As shown in Fig. 12–1, a surgical thread was sewn into the tip of tongue and the tongue was stretched with various loads through a pulley system. Movement of tongue was recorded by a force displacement transducer (FTO 3, Grass Instrument Co.).

Since possible existence of afferent nerve fibers in the lingual and glossopharyngeal nerves in the cat has been reported[1,13,21] these nerves were cut off bilaterally in advance in the experiments to prevent intermingling of lingual and glossopharyngeal effects.

RESULTS AND DISCUSSION

1. *Interrelations between tongue and mandibular movements.*

As shown in Fig. 12–2A., when the mouth of a cat was opened in a supine position, the tongue of the cat contracted and increased its tension, and the tip of the tongue also bent to the floor of the mouth. This tongue action was induced even by jaw opening of only 1 cm. That this response is an active physiologic reflex phenomenon, and not a passively induced movement, is shown in that this tongue action disappeared under deep anesthesia and after bilateral section of the hypoglossal nerve (Fig. 12–2B).

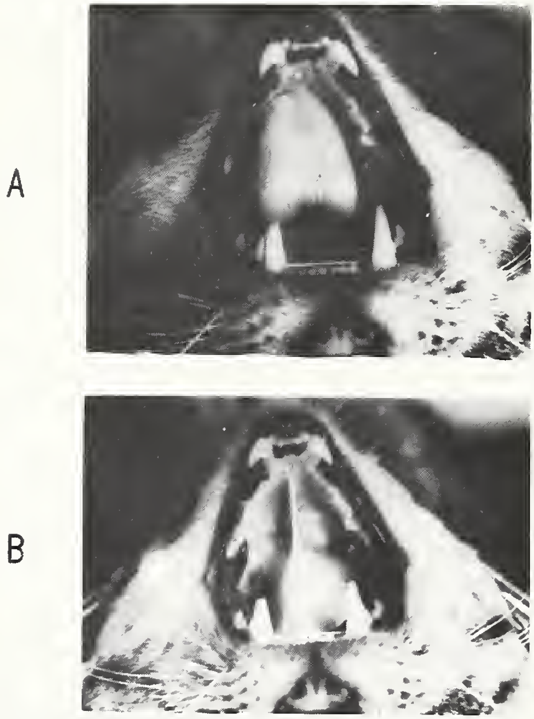

Figure 12–2. Tongue retraction and bending reflex induced by opening of the mouth with cat in supine position.
A: under normal conditions
B: after bilateral section of the hypoglossal nerve

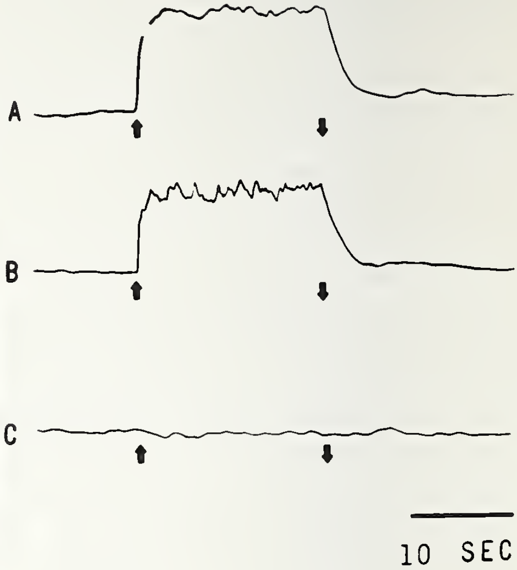

Figure 12–3. Summated EMG activities of tongue muscles under mouth opened condition.
A: styloglossus muscle
B: genioglossus muscle
C: hyoglossus muscle

During passive mouth opening with 500g load, as shown in Fig. 12–3, prominent EMG activity was induced in the styloglossus and genioglossus muscles, but the hyoglossus muscle showed no EMG activity. Blom[2] reported the EMG activities of the hyoglossus muscle of a cat during jaw opening. However, since muscle fibers of the hyoglossus muscle of a cat intermingle with those in the peripheral part of the styloglossus muscle, the discrepancy of our findings from those of Blom apropos of activity of the hyoglossus muscle may depend upon the site of the recording electrode. In the EMG response in the styloglossus and genioglossus muscles, there was no dynamic phase at the beginning of the response, and the summated activities of these two muscles was maintained almost constant in level while the mouth was opened by a constant load. The EMG responses of the jaw closing mus-

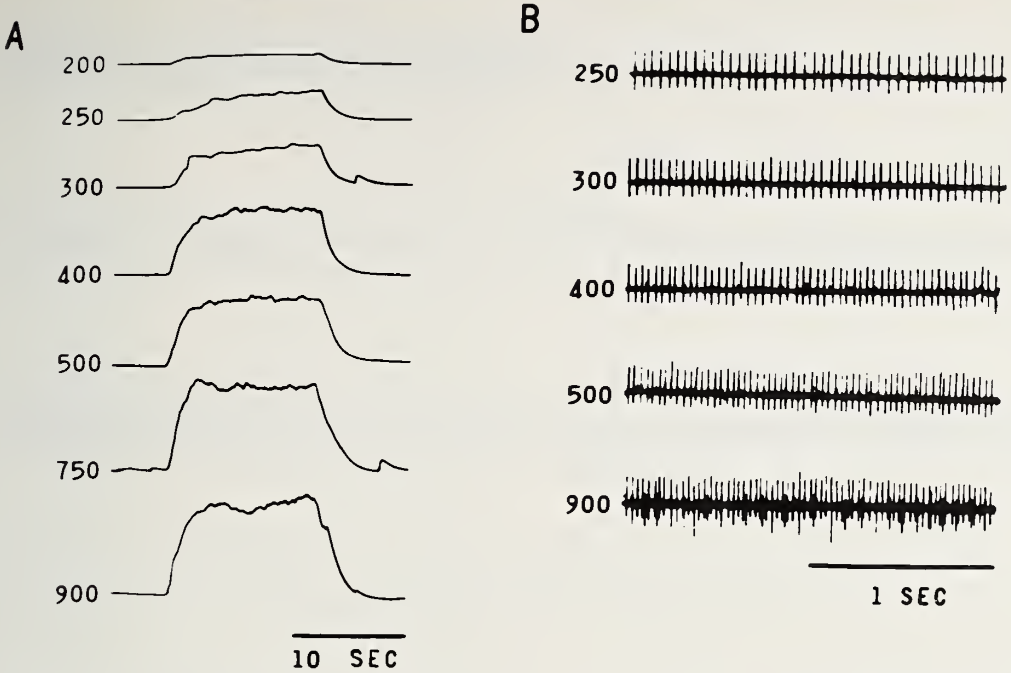

Figure 12–4. Relations between degree of jaw opening and tongue muscle activity.

A: Summated EMG activity of the styloglossus muscle

B: single motor-unit response of the styloglossus muscle .
Numerals indicate weights of load used for depression of the mandible.

cles during jaw opening have initial high burst discharges: the dynamic phase. This initial response decreases to a steady state at a certain level within a short time, while the jaw is drawn open. This pattern of EMG responses of the jaw closing muscle can possible be attributed to afferent impulses via Ia fibers from the muscle spindle of the jaw closing muscle itself. However, considering the absence of a dynamic phase in the EMG patterns, the EMG activities of the styloglossus and genioglossus muscles may be activated by some slow adapting afferent impulses. The degree of such EMG activation of styloglossus and genioglossus muscles during mouth opening was proportional to the load depressing the mandible.

As shown in the left side of Fig. 12–4, as a sample record, the integrated EMG activities reached their peak level. Analysis of the unit EMG discharges of this response of the styloglossus muscle in the same animal revealed that some of the unit EMG composing the summated activities began to fire at about 150gm load, as shown in the right side of the figure, and most of the units fired with 250–400gm load. Interrelations between the integrated EMG activities and the unit EMG activity of the same styloglossus muscle are illustrated in Fig. 12–5. The unit which began to fire with a lower load (a lower threshold unit) reached the maximum level of firing rate earlier than that of a higher threshold unit. In Fig. 12–5, the open circles show the magnitude of summated EMG activities, and the solid circles show the unit discharges of the same styloglossus muscle. The abscissa of the figure is weight of the load, the ordinate in the left side shows magnitude of summated

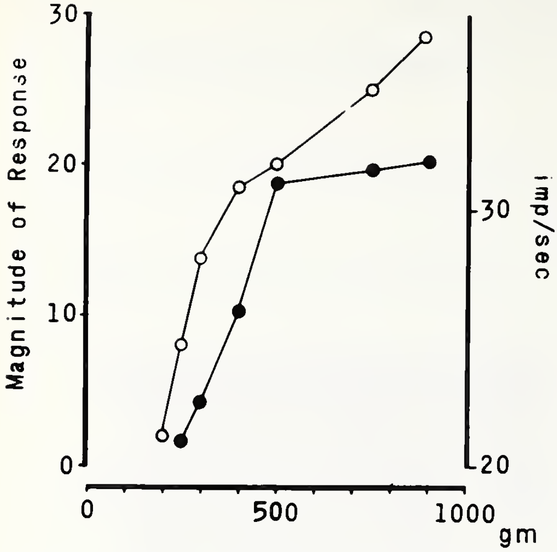

Figure 12–5. Relations between summated EMG activities and single unit activities of the styloglossal muscle.
solid circles: unit activity
open circles: summated response
abscissa: load (gm)
ordinate (left): magnitude of summated response
ordinate (right): frequency of unit activity

EMG activities and the ordinate in the right side shows frequencies of the unit EMG discharge of the muscle. The unit illustrated in this figure reached maximum activity level with 500 gm load, but the summated activities continued to increase to 1.0 kg load. This means that other units with higher threshold increased firing even after some units have reached their maximal level of activity with 500gm load.

This reflex tongue activity under jaw opening was not influenced by bilateral section of the masseter and temporalis nerves. This fact suggests that afferent impulses from the masseter and temporalis muscles may not contribute to this reflex. In addition, bilateral removal of temporomandibular joints did not at all affect this reflex. However, this reflex was completely abolished after bilateral section of the medial pterygoid nerves. Therefore, some afferent impulses from the medial pterygoid muscle via the medial pteryoid nerve may well contribute to this reflex.

In the next step of the experiment, a repetitive pulse stimulation was applied to the medial pterygoid nerve and the response of functional single fiber in the hypoglossal nerve was recorded. Spontaneous discharges of the hypoglossal nerve were activated when the frequency of stimulation was from 20 Hz up to 40 to 100 Hz. In Fig. 12–6, A is the control record without stimulation and it shows periodical firing in response to respiratory phases. Stimulation of the medial pterygoid nerve with 60 Hz induced firing of some hypoglossal nerve fibers. The discharges of the hypoglossal nerve fibers were irregular and had no relation to frequency of the stimulating pulse, and a single pulse stimulation induced no such responses in the hypoglossal nerve. These experimental facts suggest that the reflex pathway of this tongue response by mouth opening is

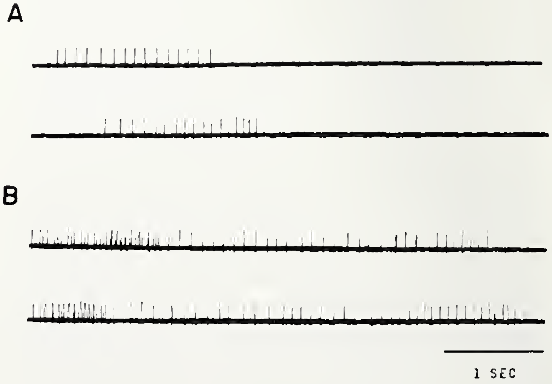

Figure 12–6. Response of single unit of the hypoglossal nerve induced by stimulation of the medial pterygoid nerve.
A: control, with periodical firing with inspiration
B: activation during stimulation by 60 Hz pulse

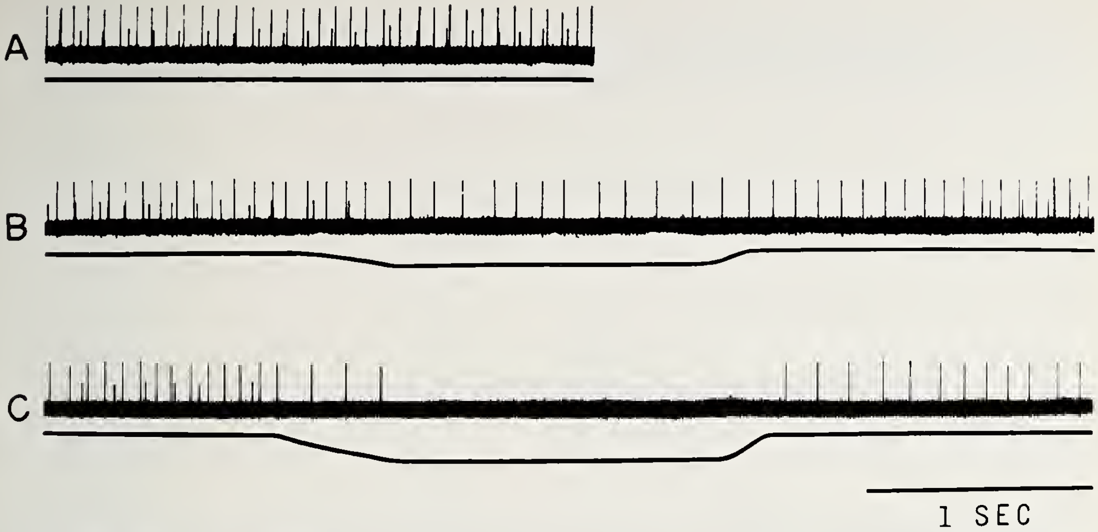

Figure 12–7. Inhibition of spontaneous firing in efferent nerve fibers of the medial branch of the hypoglossal nerve induced by forward stretch of the tongue.
A: control
B: weak stretch
C: strong stretch
The upper trace is an action potential and the lower is mechanogram of tongue stretch.

not simple. Afferent impulses via the medial pterygoid nerve concerned with this response are assumed to be transmitted to the hypoglossal motor nucleus via interneurons and, after summation and modification of impulses in some place in the lower central nervous system, they may be transmitted to the hypoglossal motoneurons, there to facilitate the action of tongue retraction and bending of the tongue tip to the floor of the mouth.

2. *Autonomous control of tongue muscle activities.*

Some of the hypoglossal nerve fibers in the medial branch showed regular spontaneous discharges, as in Fig. 12–7A. Spontaneous discharges of about one third of these examined units were inhibited by forward stretch of the tongue, but discharges of the two thirds of the units were not affected at all by the procedure.

As shown in Fig. 12–7B, weak forward stretch of the tongue resulted in inhibition of a unit showing low amplitude discharge and decreased rate of the discharge of a higher amplitude unit. However, stretch of the tongue by stronger force inhibited almost all of these discharges (Fig. 12–7C). Fig. 12–8 shows decrease in the firing frequencies in a sample nerve fiber from regular spontaneous discharges at about 14 Hz, proportional to increase of the load stretching the tongue. In most cases, the inhibitory response appeared at 20–30gm load for tongue stretch and discharges were completely inhibited at 200gm load. No nerve fibers in the lateral branch of the hypoglossal nerve increased their spontaneous discharges by this procedure of forward stretch of the tongue.

This inhibition of spontaneous dis-

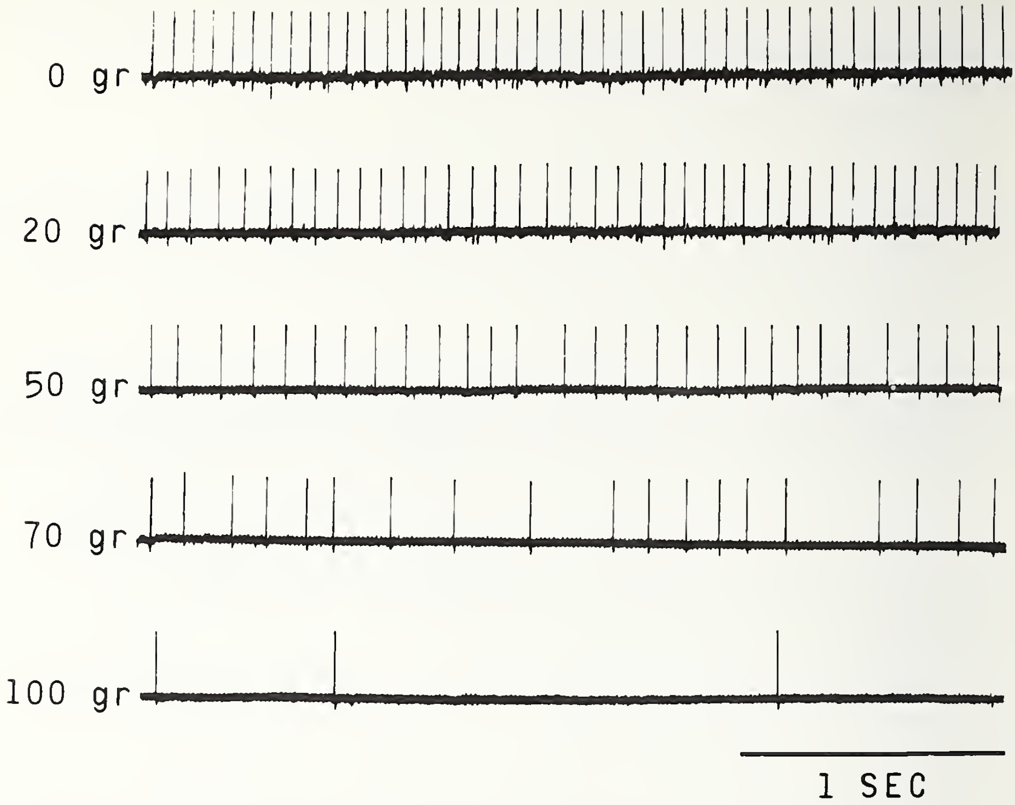

Figure 12–8. Single unit analysis of the inhibitory response of the medial branch induced by forward stretch of the tongue. Demonstration of relations between load and degree of inhibition.

charges of some nerve fibers in the medial branch of the hypoglossal nerve by tongue stretch was eliminated after section of the lateral branch of the hypoglossal nerve. This phenomenon is also very sensitive to anesthesia; the inhibitory response was not induced in deeper anesthesia.

The relation between the discharge frequencies of an afferent nerve fiber in the lateral branch and those of an efferent nerve fiber in the medial branch are illustrated in Fig. 12–9. In this Figure, single fiber activity with tongue stretch in the lateral branch is indicated by open circles and that in the medial branch by solid circles. Spontaneous discharge in the efferent fiber, the medial branch, at about 14 Hz was slightly inhibited to 12.7 Hz by tongue stretch with 20gm load. Following increase of the load to 100gm, the spontaneous discharges in this nerve fiber were linearly inhibited. The afferent unit, in the lateral branch, began to discharge at tongue stretch with 10gm load; following increase of the load to 50gm, discharges were linearly increased and reached the maximal level of discharge at 100–200gm load. Spontaneous discharges of the efferent nerve in the medial branch corresponding to these afferent nerve discharges were inhibited.

These experimental results strongly suggest that some afferent information from the tongue via the lateral branch of the hypoglossal nerve inhibits activity of some tongue muscles which are acti-

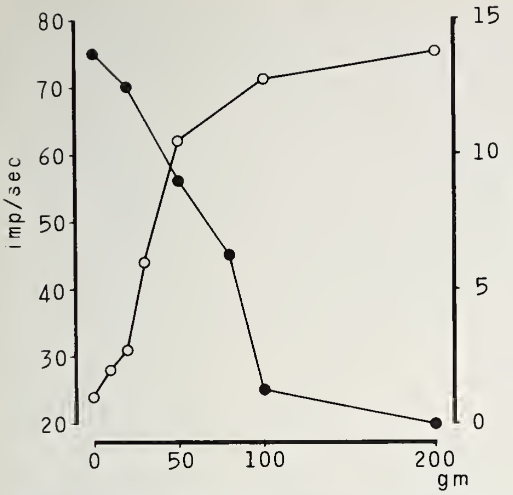

Figure 12–9. Relation between inhibition of firing of a unit in the medial branch (efferent) and activation of firing of a unit in the lateral branch (afferent) of the hypoglossal nerve.
open circles: firing rate of unit in lateral branch
solid circles: inhibition of firing of unit in medial branch
abscissa: load of tongue stretch
ordinate (left): firing rate of open circles
ordinate (right): firing rate of solid circles

Control firing with 14 Hz of unit in the medial branch was decreased to about 12.7 Hz by stretch of the tongue with 20 gm load. Following increase of degree of tongue stretch up to 100 gm, inhibition of firing rate was linearly increased. Conversely, firing rate of a unit in the lateral branch was activated by forward stretch of the tongue. This activation was induced by weak tongue stretch, with 10 gm load, and this activation increased linearly up to 50 gm. The firing reached maximal level at 100-200 gm load.

vated through impulses via the motor nerve in the medial branch of the hypoglossal nerve. In a previous paper, Morimoto and Kawamura[19] showed that single pulse stimulation to the hypoglossal nerve induced IPSPs in most of the hypoglossal montoneurons and speculated about possible contribution of some afferent impulses via the hypoglossal nerve in the response. However, the physiologic properties of these afferent fibers in the hypoglossal nerve had not been evaluated. The present experiments demonstrate that the hypoglossal afferent impulses from some tongue muscle stretch receptors induce IPSPs of the hypoglossal neurons. Blom[2] recognized no activation of EMG of the tongue muscles by tongue stretch. The background of this phenomenon is well explained by our present studies showing inhibition of tongue muscle activity from afferent impulses from the stretch receptors of the tongue. This experiment demonstrates that some afferent impulses via the lateral branch of the hypoglossal nerve inhibited some motoneurons in the hypoglossal nucleus, a phenomenon which corresponds to the relation between extensor and flexor limb muscles. The relation between afferent and efferent impulses via the lateral branch of the hypoglossal nerve were not evaluated in the present experiment because of technical difficulty. The threshold of tongue stretch inducing discharges of the afferent fibers in the lateral branch was lower than that inducing inhibition of the efferent fiber discharges in the medial branch. This fact suggests that the afferent impulses will be spatially and temporally summated at some interneurons in the lower medulla and may have an inhibitory effect on the hypoglossal motoneurons.

SUMMARY

Tongue retraction and tongue tip bending to the floor of the mouth were reflexly induced by passive jaw opening. This reflex was elicited by afferent impulses via the medial pterygoid nerve. The styloglossus and geniglossus

muscles contributed to this tongue action. Impulses via the temporal and masseter nerves were not related to this tongue reflex. These observations strongly suggest that afferent impulses from the medial pterygoid muscle play some role in the delicate coordination of lingual and mandibular muscle activities.

These experiments have also demonstrated another mechanism of autonomous control of tongue function: the inhibition of tongue protrusive muscles, innervated by the medial branch of the hypoglossal nerve, by forward stretch of the tongue, eliciting afferent activity in the lateral branch.

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Chapter 13

RESPONSES OF HYPOGLOSSAL MONONEURONS TO TRIGEMINAL AFFERENTS IN THE TRIGEMINO-HYPOGLOSSAL REFLEX

Rhyuji Sumino and Yoshio Nakamura

In order to reveal the role of trigeminal afferents in the regulation of tongue movement, the synaptic response of hypoglossal motoneurons to stimulation of the inferior alveolar nerve and the masseter nerve has been studied in adult cats either anesthetized with sodium pentobarbital or decerebrated. For intracellular recording, glass micropipettes filled with 3 M KCI solution were used.

The synaptic potentials in hypoglossal motoneurons, induced by stimulation of the inferior alveolar nerve were EPSP-IPSP sequences or IPSPs (Fig. 13–1). The thresholds of these synaptic potentials were very low, always less than 1.2 times of the nerve threshold. The minimum latency of EPSPs was 1.6 msec, indicating that an excitatory disynaptic linkage was involved in this reflex arc.

These findings are similar to those of synaptic potentials in the hypoglossal motoneuron which were produced by lingual nerve stimulation.[11] Functionally, the low-threshold afferents of either nerve induce a jaw-opening reflex, and the large diameter fibres in the inferior alveolar nerve are correlated with the tactile and pressure sensation of the periodontal and mucous membranes around the tongue.[10] Therefore, these data suggest that afferents from mechanoreceptors around the tongue as well as those on the surface of the tongue[12] participate in regulating ton-

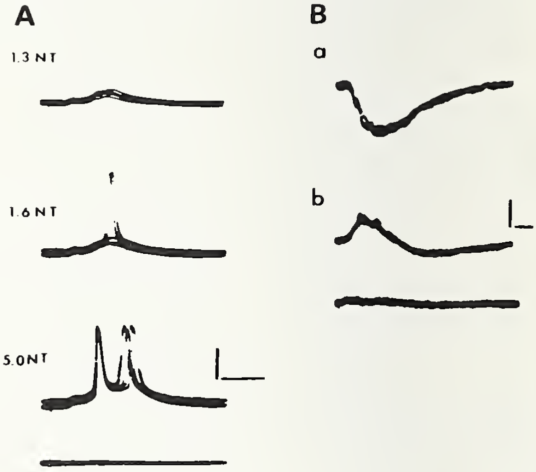

Figure 13–1. Synaptic potentials of hypoglossal motoneurons produced by stimulation of the ipsilateral inferior alveolar nerve.

A. Depolarizing synaptic potentials (IPSPs) with one or two spikes in a hypoglossal motoneuron. Numbers to the left indicate stimulus intensities in multiples of the nerve threshold (X N.T.). Voltage calibration: 20mV. Time calibration: 5msec.

B. Hyperpolarizing potentials (IPSPs) in another hypoglossal motoneuron; (a) Original hyperpolarizing potential; (b) Reversal of the original hyperpolarizing potential during recording with a KC1-filled electrode indicating that the original one is an IPSP. Voltage calibration: 2mV. Time calibration: 5msec.

Bottom traces of A and B represent the extracellular field potentials.

gue musculature during jaw-movement.

Morimoto, Takata and Kawamura[7] reported that stimulation of the lingual nerve evoked EPSPs in hypoglossal motoneurons which innervated the tongue retractive muscles and IPSPs in hypoglossal motoneurons which innervated the tongue protrusive muscles. In our study, however, by stimulation of the inferior alveolar nerve, EPSPs and IPSPs were evoked in both tongue retractive and protrusive motoneurons. There was no significant difference in the distribution of EPSPs and IPSPs. In addition, stimulation of the inferior alveolar nerve evoked reflex discharges in all branches of the hypoglossal nerve (Fig. 13–3 AL-M, AL-L). Hypoglossal nerve trunk was divided into the medial and the lateral branches. The former contain the nerves to the medial end branch (intrinsic tongue muscles) and the tongue protrusive muscles (genioglossus and geniohyoideus). The latter innervate the tongue retractive muscles (hyoglossus and styloglossus). These results suggest that the oral afferents travelling in the inferior alveolar nerve do not produce reciprocal effect upon the tongue retractive and protrusive motoneurons.

Recently, Morimoto, Takata and Kawamura[8] demonstrated IPSPs in hypoglossal motoneurons, both retractive and protrusive, following application of a single shock to the masseter nerve. In our studies, in addition to IPSPs, EPSP-IPSP sequences were also recorded in a number of hypoglossal motoneurons after stimulation of the masseter nerve (Fig. 13–2). This sequence was confined to the motoneurons which send their axons to the medial branches of the hypoglossal nerve. It is interesting that by stimulating the inferior alveolar nerve, EPSP-IPSP sequences were also evoked in the same hypoglossal motoneurons. Furthermore, as shown in Fig. 13–3 MA-L, reflex discharges were recorded only in the medial end branches of the hypoglossal

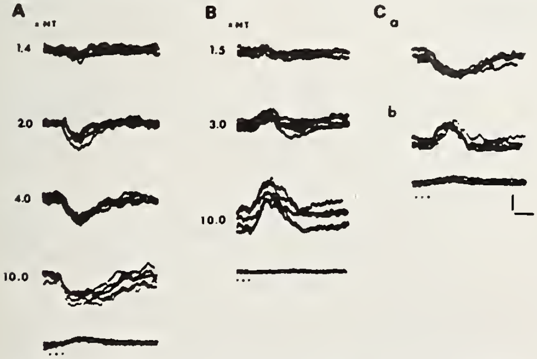

Figure 13–2. Synaptic potentials of hypoglossal motoneurons produced by triple pulses at 2.5 msec intervals to the ipsilateral masseter nerve.

A. Hyperpolarizing potentials (IPSPs) in terms of intensities of masseter nerve stimulation. Number to the left indicates stimulus intensities (X N.T.).
B. Depolarizing potentials (EPSPs).
C. Hyperpolarizing potentials before (a.) and after (b.) reversal with CF injection.

Voltage calibration: 2 mv. Time calibration: 10 msec. All bottom traces in A and B are extracellular field potentials. Three points beneath each column indicate timing of triple shocks to the masseter nerve.

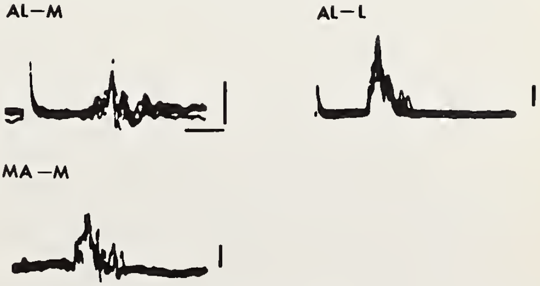

Figure 13–3. Reflex discharges of the medial end branch (M) of the hypoglossal nerve to the styloglossal muscle (L) induced by supramaximal stimulation (10 X N.T.) of the ipsilateral inferior alveolar nerve (AL) and the ipsilateral masseter nerve (MA). Voltage calibrations: 100 μV Time calibration: 2 msec for records in AL-M and AL-L, 5 msec in MA-M. Negativity is shown upwards.

nerve, which move the tip of the tongue slightly downward. These results confirm the existence of the excitatory effect of masseter afferents on motoneurons and suggest that the motoneurons which send their axons to the medial end branch were excited during the jaw-opening and jaw-closing reflex.

It is known that cell bodies of afferent fibres from muscle spindles in the masseter muscles are located in the trigeminal mesencephalic nucleus.[1,2,4,14,15] Szentágothai[14] and Mizuno and Sauerland[6] described some central processes of the trigeminal mesencephalic neurons that terminated in the hypoglossal nucleus and suggested that these proprioceptive fibres, Probst's tract, might be important in regulating tongue musculature in mastication.

In the present work, however, the latency of the synaptic potentials produced by masseter nerve stimulation was very long (6.80 ± 0.97 msec for IPSPs, 7.32 ± 1.73 msec for EPSPs) and the synaptic potentials were evoked only by stimulation of high threshold afferents, more than 1.4 X N.T. in the masseter nerve.[8]

In addition, direct stimulation of the trigeminal mesencephalic nucleus at the threshold intensity for the masseteric monosynaptic reflex[3,5,9] did not produce any synaptic potentials in the hypoglossal motoneuron.

Hence, these results indicate that muscle spindle afferents, Group Ia, in the masseter nerve do not contribute appreciably to the production of the synaptic potential in the hypoglossal motoneuron.

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Chapter 14

CONTROL OF TONGUE AND FACIAL MOTILITY: NEURAL MECHANISMS THAT MAY CONTRIBUTE TO MOVEMENTS SUCH AS SWALLOWING AND SUCKING

Barry J. Sessle and David J. Kenny

INTRODUCTION

Although there has been much behavioural and psychophysical investigation of oral, pharyngeal and laryngeal sensorimotor functions, the neural mechanisms contributing to these functions still remain largely uninvestigated. Highly coordinated movements of the lips, jaw and tongue are integral parts of oral-facial behavior and are manifested as early as the third trimester of fetal development.[11] Since sensory information from the oral-facial and pharyngolaryngeal areas may be implicated in the complex interactions involved in oral-facial activities such as sucking, swallowing, gagging, mastication, airway maintenance and speech, we have initiated a series of experiments to investigate the reflex activity contributing to the facial and lingual motility patterns involved in these oral-facial motor functions. Techniques of whole nerve compound action potential recording, electromyography (EMG), and extracellular microelectrode recording from single brain stem neurones have been utilized.

TONGUE REFLEX ACTIVITY

The methods employed have been described elsewhere[27,28] but will be outlined briefly. In anaesthetized (sodium thiamylal or chloralose) or decerebrated adult cats in which blood pressure, expired pCO_2, and rectal temperature were monitored continuously, the electromyographic (EMG) activity of the anterior intrinsic tongue muscles and anterior fibres of the genioglossus and the neural activity of the hypoglossal nerve (XII) were recorded, amplified, and displayed on an oscilloscope (Fig. 14–1A). A mechanical stimulus delivered to the larynx or a single pulse (0.01–5.0 mA, 0.1 msec) applied to the ipsilateral superior laryngeal nerve (SLN) produced a lingual protrusion that was associated with

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some curling upwards of the lateral surface of the tongue. These observations indicate activation of genioglossus and anterior intrinsic tongue muscles. Tongue EMG and XII recordings verified the presence of the observed tongue

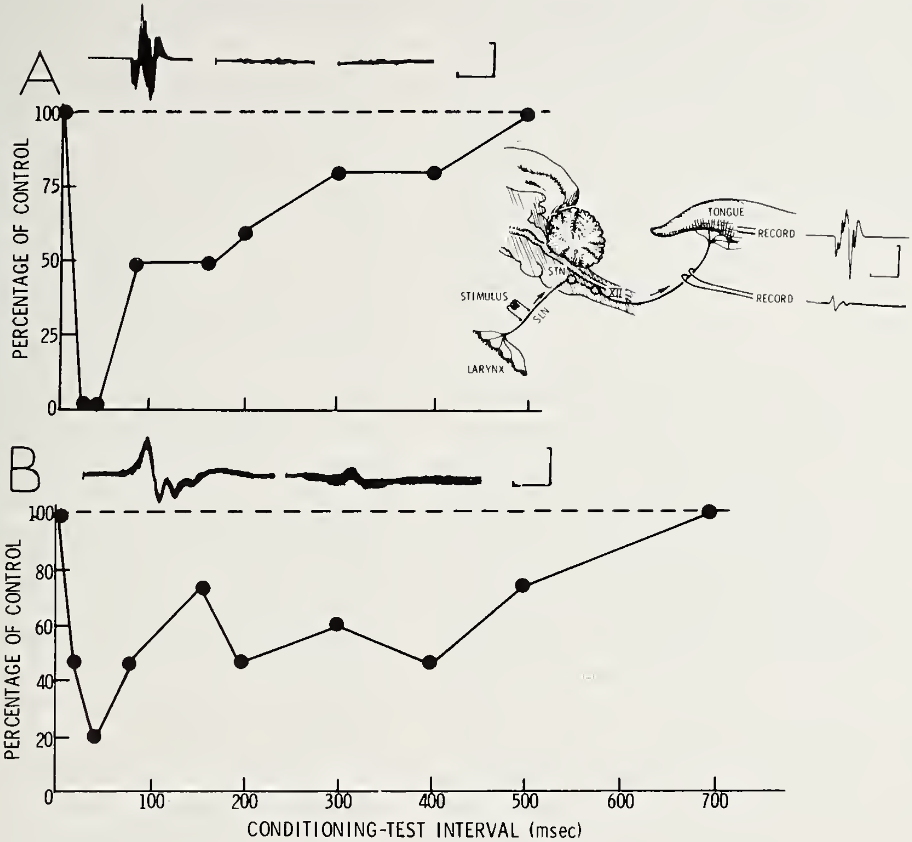

Figure 14–1. Reflex responses evoked in the tongue (EMG) and hypoglossal nerve (XII) and the effects of various oral-facial conditioning stimuli. In A, the method of recording the EMG and XII reflex responses evoked by a superior laryngeal nerve (SLN) stimulus is shown on the right, with representative records. In many cats, a bilateral tongue reflex could be produced by the SLN stimulus, which often evoked a later (approximately 25 msec latency) response as well. The three records above the graph in A show five superimposed EMG responses to five successive SLN stimuli (left record), and the inhibitory effects at a 40 msec conditioning-test interval, of glossopharyngeal (IX) nerve (middle record) and infraorbital (X) nerve (right record) conditioning stimuli. The time course of the inhibitory effect of IX conditioning stimulation is shown in the graph and was obtained by comparing, at various conditioning-test intervals, the test (control) responses to five successive SLN stimuli with the responses elicited in the presence of IX conditioning stimuli. Occasionally, a short (20 msec) facilitatory effect preceded the inhibition (not shown). The graph in B similarly illustrates the time course of inhibition produced by SLN conditioning stimulation of the response evoked in XII by a lingual nerve stimulus. The left record shows five superimposed responses to five successive lingual nerve stimuli, and the right record the inhibition of the responses at a conditioning-test interval of 20 msec. Voltage calibration: 0.4 mV for graph records in A, 0.1 mV for B; time calibration: 10 msec. For method records in A, voltage calibration: 0.4 mV (upper), 0.2 mV (lower); time calibration: 10 msec for upper and lower records.

movements and established their reproducibility (Fig. 14–1A). Intravenous administration of the muscle relaxant, gallamine triethiodide, abolished the EMG response but not the XII response. The EMG responses to SLN stimulation had a latency of 11.0 ± 2.14 msec (mean ± standard deviation) and a stimulus threshold of approximately 0.6 mA, which is comparable to the SLN stimulus intensity required for activating laryngeal mechanoreceptive afferent fibres.[29]

A tongue reflex was not elicited from any of the other nerves or regions stimulated in this study (see below) with the exception of the anterior digastric nerve (but only at more than five times its threshold of 0.6 mA for producing digastric contraction), and the lingual and glossopharyngeal (IX) nerves. However, in contrast to the laryngeal-evoked tongue protrusion, low-intensity stimulation of the lingual nerve or IX produced predominantly a retrusive tongue reflex movement. Such reflex activity may contribute to lingual movement patterns in man. For example, a tactile stimulus to the tongue of the neonate produces observable tongue movements.[41]

Our observation of a short-latency, low-threshold tongue protrusive reflex evoked by a laryngeal stimulus is compatible with reports[36,37] that SLN stimulation may activate cat hypoglossal motoneurones with a 4–12 msec latency. The tongue protrusive reflex does not show[27] all the characteristics of an 'elementary' reflex,[5] but still may be implicated in functions such as coughing and airway protection as suggested[5] for the elementary reflexes. We have also noted[27] that tongue protrusion may precede the swallow synergy in cat (cf[5,6]), and genioglossal muscle activity occurs in the human swallow.[10] This would suggest then that the neural mechanisms underlying the tongue protrusive reflex may be brought into play during deglutition.

The laryngeal-evoked reflex may also provide neural processes that contribute to the control of tongue position and motility in other aspects of normal and abnormal oral-facial function. Because of this, we decided to determine if the reflex could be regulated from other oral-facial regions that might also be involved in coordination of tongue movements. The SLN 'test' stimulus, which produced the reflex response in the anterior tongue musculature or XII, was preceded at various time intervals by a conditioning stimulus (single pulse, 0.01–5.0 mA, 0.1 msec) delivered to the infraorbital nerve (I0), IX, SLN, lingual, masseter and digastric nerves. In some instances, an electrical stimulus to the forepaw or mechanical stimulus[30] to the maxillary canine was applied. At each conditioning-test interval, the amplitude of the responses to five or more successive SLN stimuli was compared with that of the responses in the presence of a conditioning stimulus.

Conditioning stimulation of IX, SLN or lingual nerve was particularly effective in inhibiting the laryngeal-evoked response in the tongue or XII. The inhibition started at a conditioning-test interval of approximately 10 msec, reached its peak at 20 to 40 msec, and usually lasted for 400 msec or more (Fig. 14–1A). Electrical stimulation of the upper lip or IO and innocuous mechanical stimulation of the canine had an inhibitory influence (Fig. 14–1A) of 150 msec duration, although occasionally it did last for as long as 400 msec. No conditioning effects were obtained by forepaw stimulation but jaw muscle afferent stimulation resulted in facilitatory and/or inhibitory influences[27] that usually required stimuli that were at least five times threshold (cf[21,28]).

As well as having an excitatory influence on the tongue, SLN stimulation also exerted an inhibitory influence on other excitatory inputs to the tongue. For example, prior laryngeal stimulation had a prolonged inhibitory effect on responses evoked in XII by lingual nerve or IX stimulation (Fig. 14–1B). These responses could also be regulated by conditioning stimulation of lingual, IX, X, tooth and jaw muscle afferents.

The excitatory and inhibitory interactions on tongue motor activity, some of which have been described by others[2,20,21,22,23,25,37,38], provide a neural integrative basis for the coordination of tongue motility with other oral-facial movements. For example, the inhibitory influence from the tooth on tongue protrusion may constitute a protective mechanism for the tongue during mastication, similar to that suggested[25] for influences from jaw muscle afferents. It may also serve to depress tongue thrusting during swallowing. Eruption of the teeth and their subsequent contact with opposing teeth, tongue, lips, etc, could signal the change over from an infantile to an adult pattern of swallowing. Children with malocclusions in which the maxillary and mandibular anterior teeth no longer contact each other may show a tongue protrusion during swallowing[35] which also occurs during the period when the primary anterior teeth have been shed and the permanent teeth have not yet erupted.[19] After the secondary maxillary incisors erupt, the protrusion is no longer apparent. It is also noteworthy that the periodontal tooth-supporting structures become innervated only after eruption of the primary or permanent teeth into the oral cavity[1] and so regulatory influences from periodontal receptors would only then become operational. Thus, it seems possible that the regulatory mechanisms from the tooth observed in the present study might underly these clinical observations of tongue protrusive swallowing and its regression.

Previous studies[20,21,22,23,37,38] have reported that hypoglossal neurones may be inhibited by stimulation of lingual, IX, X, SLN and high-threshold jaw muscle afferents. The duration of the hypoglossal inhibitory postsynaptic potentials produced by this stimulation is rarely more than 100 msec and thus much shorter in duration than the inhibitory effects observed in our studies. The long time course of inhibition of the tongue protrusive reflex correlates very closely in onset, peak and duration with the time course of inhibition produced by these oral-facial afferents in brain stem neurones in the solitary tract nucleus.[28,31] For example, Fig. 14–2 shows the typical long duration of inhibition produced by conditioning stimulation of SLN and IX in single solitary tract neurones with laryngeal (Fig. 14–2A) or pharyngeal (Fig. 14–2B) mechanoreceptive fields. These findings indicate that, in addition to postsynaptic inhibitory influences on hypoglossal motoneurones, inhibitory mechanisms operating at the first synaptic relay of oral, pharyngeal and laryngeal sensory information (the solitary tract nucleus) constitute a significant factor in the depression of tongue reflex activity. Presynaptic regulatory influences may account for much of the inhibitory effects observed in the solitary tract nucleus.[12,26,29,31]

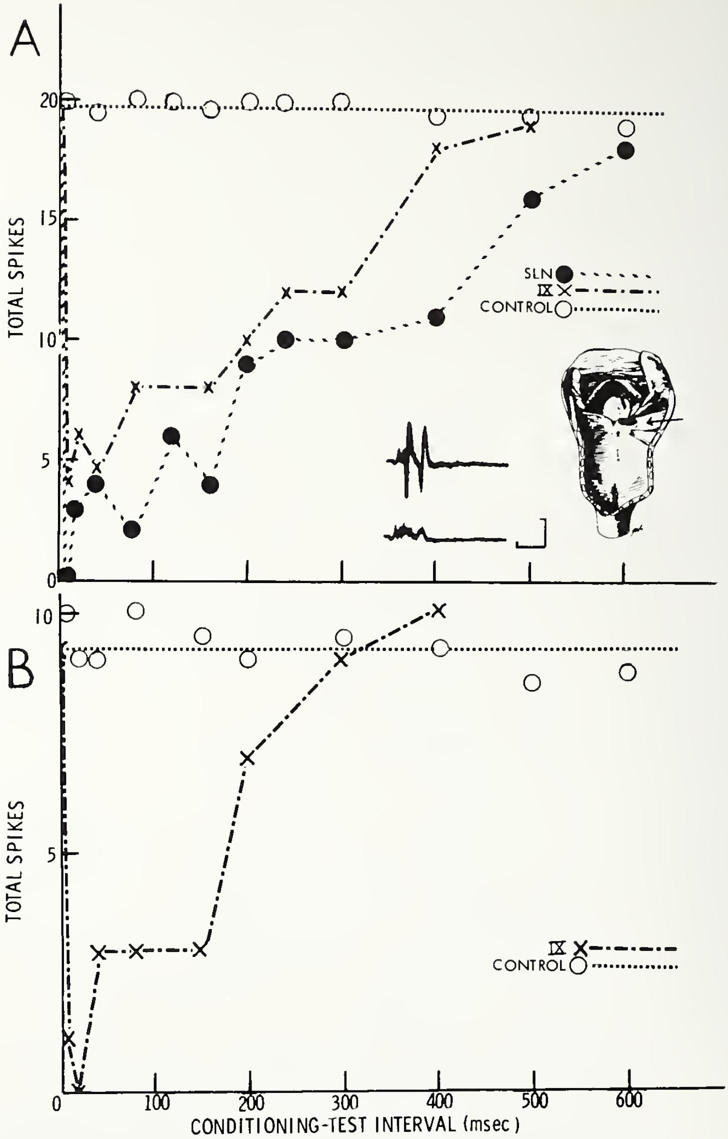

Figure 14–2. Time courses of inhibition of single neurones in the solitary tract nucleus activated by superior laryngeal (SLN) and glossopharyngeal (IX) nerve stimulation and conditioned by SLN and IX stimuli. In A, the upper record shows five superimposed responses of a solitary tract neurone to five successive SLN stimuli and the lower record illustrates the inhibitory effect, at a 40 msec conditioning-test interval, of SLN conditioning stimulation. The neurone's laryngeal mechanoreceptive field is also shown (arrow) by the black area in the illustration of the exposed larynx. The time course of inhibition produced by SLN and IX conditioning stimulation as shown in the graph was obtained by comparing, at various conditioning-test intervals, the total number of spikes evoked by 10 SLN test stimuli (control) with the number obtained by 10 stimuli in the presence of a conditioning stimulus. B similarly illustrates the time course of inhibition produced by conditioning stimulation of the responses evoked by IX stimuli in another solitary tract neurone with an oropharyngeal receptive field. Voltage calibration: 0.5 mV; time calibration: 5 msec.

FACIAL REFLEX ACTIVITY

Methods similar to those described above were used for recording the EMG activity of the perioral facial musculature (caninus and quadratus labii superioris) and the neural activity of the facial nerve (VII). Responses could be evoked in the facial musculature and VII by a low-intensity stimulus to X, IX and SLN (Fig. 14–3). The EMG response, but not the VII response, could be abolished by gallamine thiethiodide administration.

Mean latencies for the ipsilateral EMG responses were 13.6 ± 7.82 msec (X), 19.1 ± 6.71 msec (IX) and 27.8 ± 5.71 msec (SLN). Facial reflex activity evoked with a comparable latency by stimulating X, or the skin that it innervates, has been previously reported in cat[18,40] and in man.[7,14] Innocuous stimulation of the anterior tongue in man is also effective.[3] However, a long-latency response is elicited by noxious intraoral stimuli,[3] stimulation of high threshold VII and XII afferents,[9,17,18] and low-intensity stimulation of the trigeminal area.[7,14,18] The responses we noted with IX and SLN stimulation had a similarly longlatency, but the low threshold (<1.0 mA) of the response is indicative of activation of mechanoreceptive afferents.[29]

The facial reflex activity evoked by X stimulation may be related to observations that tactile stimulation of the perioral area initiates facial movements associated with sucking and the rooting reflex in both fetus[11] and normal neonate.[24] Likewise, the facial reflexes that we observed with IX and SLN stimulation may also be involved in sucking. IX and SLN neural activity can influence facial motility in the human neonate (Steiner, Chap. 17, this book). Moreover, differences in the amount of pharyngeal and laryngeal stimulation which occur in nutritive versus non-nutritive sucking might contribute to the differences in amount and pattern of both types of sucking behaviour observed in neonates.[33,42]

Conditioning techniques similar to those described above were used to investigate regulatory influences on facial motor activity. As Fig. 14–3 shows, the response in VII evoked by a test stimulus to X can be initially facilitated, and then inhibited for more than 400 msec, by prior stimulation of IX and SLN. The facial EMG or VII response evoked by either SLN or IX could similarly be regulated by X, IX and SLN conditioning stimuli. Conditioning stimulation of VII, XII, saphenous, and radial nerves has also been shown to produce facilitatory and prolonged inhibitory effects on facial reflexes.[9,15,18]

Facial motor activity might be modulated by influences other than just those operating postsynaptically in the motor nucleus.[13,16,39] Neurones in the trigeminal brain stem sensory nuclei, which have perioral mechanoreceptive fields and may be involved in the relay of trigeminal sensory information to the facial motor nucleus,[4,34] show a long period of inhibition with pharyngeal and laryngeal conditioning stimuli.[8] This suggests that inhibitory influences on facial motility, as on tongue motility (see above), might be exerting their effect at the first synaptic sensory relay as well as on the motoneurones concerned.

A jaw-opening reflex occurs as a result of pharyngeal, laryngeal[27] and trigeminal[32] stimulation. This reflex and the tongue and facial reflexes examined in the present study would appear to

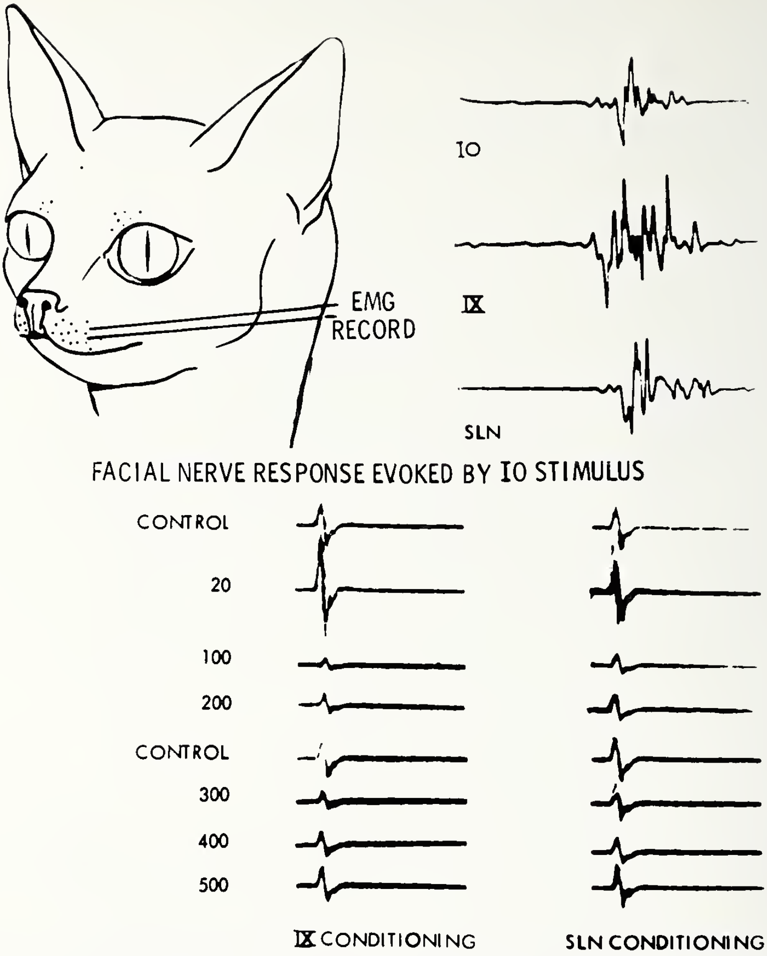

Figure 14–3. Facial EMG and nerve (VII) ipsilateral responses evoked by low-intensity stimulation of the infraorbital (X), glossopharyngeal (IX), and superior laryngeal (SLN) nerves. The upper part of the figure shows the EMG responses to single X, IX and SLN stimuli. The lower part shows, in another cat, the effects of conditioning stimulation of IX and SLN on the responses evoked in VII by X stimulation. Each record represents five superimposed responses to five successive X stimuli. The conditioning effects and their time courses are shown by the change in amplitude of the responses at various conditioning-test intervals indicated on the left. Voltage calibration: 0.5 mV; time calibration: 5 msec for records in upper part, 10 msec for records in lower part of the figure. In many cats, the X, IX and SLN stimuli elicited bilateral perioral facial responses, and X stimulation also evoked reflex activity in the obicularis oculi that could be inhibited by IX and SLN conditioning stimulation.

constitute integral parts of the multiplicity of normal and abnormal orofacial motility patterns associated with sucking, swallowing, gagging, mastication, airway maintenance and speech. It would appear to be of much value to study these reflexes ontogenetically in the primate so as to gain insight into the development of reflex activity and to provide information of use in the clinical management of feeding problems of neurologic origin associated with sucking, swallowing, gagging and mastication.

SUMMARY

Studies utilizing EMG, whole nerve, and single neurone recordings in cat have been initiated to gain more insight into the neural mechanisms underlying oral-facial motility. A laryngeal stimulus was found to produce a low-threshold reflex response in the anterior tongue musculature and in XII which was associated with a protrusive movement of the tongue. Other excitatory influences on the tongue musculature came from IX, lingual and digastric nerve afferents. We also noted that low-threshold reflex responses could be elicited in the perioral facial muscles and in VII by stimulation of IO, IX and SLN afferents.

These responses in the tongue and facial muscles were under the control of sensory information from various oral-facial areas. The time course of the prolonged inhibitory influences on the responses correlated very closely with the time course of inhibitory effects noted in single neurones in the solitary tract and trigeminal sensory nuclei. This suggests that the observed inhibitory influences on tongue and facial motility may be produced by inhibitory mechanisms operating at the first synaptic sensory relays as well as on the motoneurones concerned.

These findings are discussed in relation to their possible contribution to normal and abnormal patterns of oral-facial motility associated with functions such as swallowing and sucking.

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Chapter 15

ELICITED TONGUE MOVEMENTS: TOUCH AND TASTE IN THE MOUTH OF THE NEONATE

James M. Weiffenbach and Bradley t. thach

INTRODUCTION

The human infant was once thought capable of only gross and undifferentiated sensory perception. In recent years, this view has been successfully challenged by experimental studies of vision, audition and olfaction. These senses of the infant have not changed but better ways of studying them have been found. There have been increases in the sophistication of stimulus specification and response definition. Powerful experimental paradigms have been employed to clarify inferences from observed behavior to mechanisms of sensory integration. However, achieving adequate stimulus site specification and response definition has proved to be an unusually intractable problem for investigators of neonatal taste.

Specification of the effective site of stimulation is made difficult by the fact that fluids presented for ingestion stimulate not only taste receptors on the tongue but also receptors at other sites throughout the mouth and pharynx. Furthermore, when these fluids have passed over the taste receptors they reach the stomach where they may continue to affect ingestion behavior. The elicitation of differential amounts of ingestion or differential rates of suckle has commonly served as the basis for inferring taste discrimination.[4,8,10,12,14,17,18] Such inferences are, however, unjustified if effects due to stimulation of taste sensitive elements cannot be separated from effects due to loading the stomach with test fluids. The very differences in ingestive behavior that provide an index of taste discrimination also insure that differential stomach loads will be associated with each of the tastant conditions being compared. Sham feeding techniques whereby animal preparations can be provided oral and pharyngeal stimulation in isolation from pastric loading are not appropriate for normal human infants. However, studies of infants with gastrostomy tubes incident to recovery from reconstructive surgery indicate an effect of gastric load beyond that attributable to the ingestion process itself.[28]

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The inability to specify the site of action for ingested fluids not only prevents the separation of taste from non taste effects but also places constraints upon the description of the taste mechanisms themselves. During ingestion tastants reach an unspecified sample of the taste end organs that are distributed throughout the infant's mouth and pharynx.[13] Selective anesthesia has been used to provide gross delineation of effective stimulus sites within the mouth of the adult[6] but this procedure is not appropriate for newborn infants. Thus, within the context of the ingestion study, neither Henkin's provocative hypotheses of spatial localization of function within the oral cavity[5] nor the classical notions of localization on the tongue[21] may be tested in the newborn.

The ingestion technique is further limited in that it is inappropriate for the study of such clinically and theoretically important infants as young prematures that have not yet established suckle feeding, term infants exhibiting feeding problems and pharyngeally impaired infants.

To circumvent the problems inherent in the ingestion technique, some investigators have placed small quantities of various tastants directly on the infant's tongue.[1,19] Such a procedure provides stimulus site information, minimizes the effect of post-ingestional factors and provides access to the infant that does not or cannot suck. However, these studies, and other using larger volumes of fluids,[22,23] share the unresolved problem of adequately defining an indicator response. Perhaps because attention has been centered on discrimination between the qualitatively different submodalities of taste (sweet, sour, salty and bitter), the necessary demonstrations of a relation of stimulus to response within a given submodality have not been made. The complex mimetic or gestural responses that have been observed as responses to the local application of tastants lend themselves to analogy with the subjective responses of adults but are difficult to quantify.

A previously unexplored solution to the response definition problem is offered by the elicited lateral tongue motion. We have described a discrete lateral displacement of the tongue tip elicited by touch stimulation of the tongue.[26] Figure 15–1 illustrates elicitation of the response by nylon filament. This elicited tongue movement has much to recommend it as an indicator response for evaluation of oral sensitivity in newborns. The response is reliably observed and is specific to stimulus location, right going for right side stimulation and left going for left side stimulation. Its frequency of occurrance is an orderly function of the intensity of a touch stimulus. A standard testing routine based on this reflexly elicited movement has been used with term infants,[26] premature infants[24,25] and infants with clefts of lip and palate.[27] Some of the infants in these studies were tested as soon as 4 hours after birth. Since single drops of fluid applied to the newborn's tongue evoke ipsilateral tongue movements similar in form to tactually elicited ones it is possible that this proven indicator response can be adapted for the study of taste.

If an assessment based on a reflex elicitation of the lateral tongue movement response by drops of fluid can be developed, several advantages for the study of taste would accrue. The major reduction of test fluid volume relative to that required for an ingestion study should minimize the effects of gastric loading upon response. Since the ex-

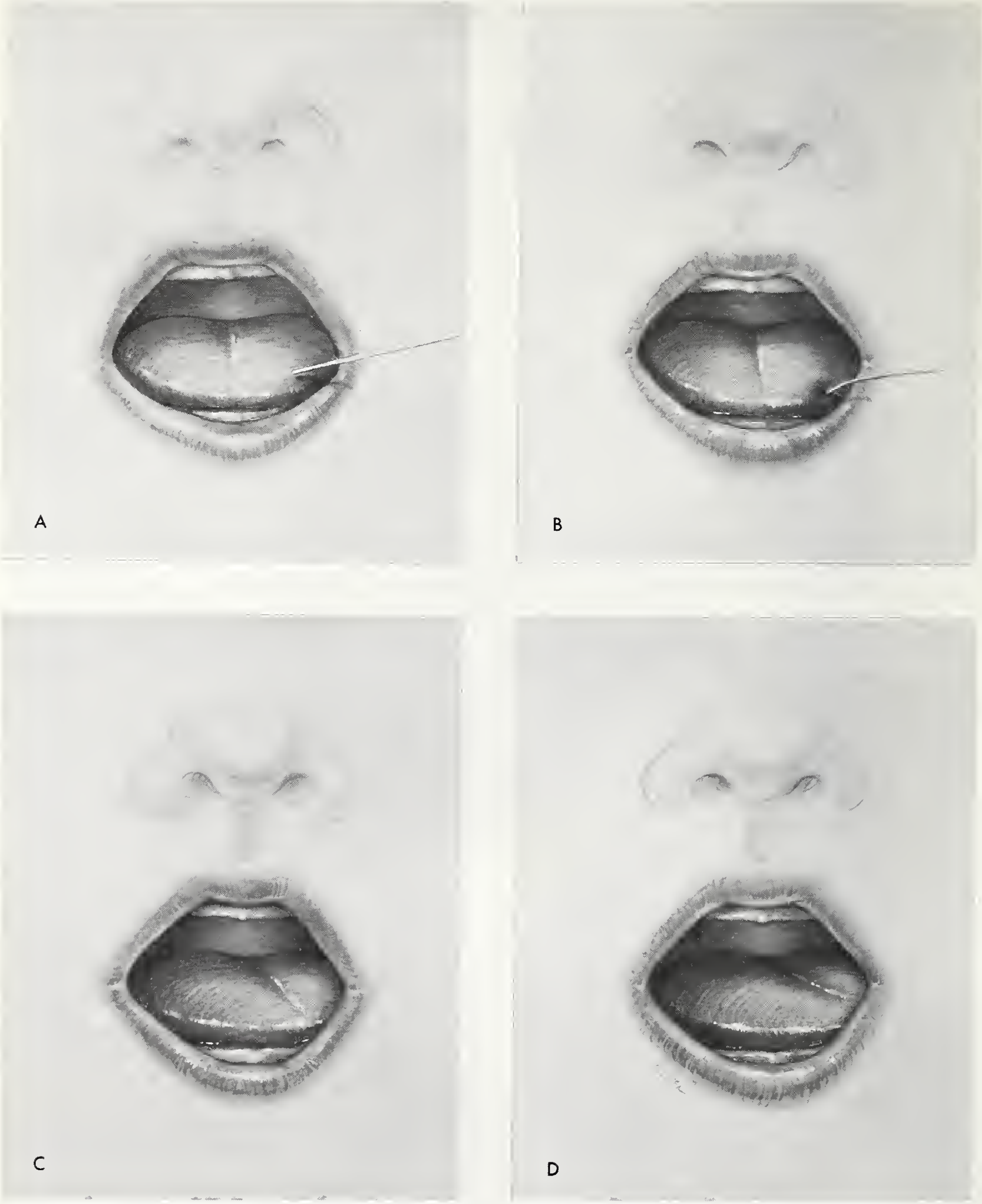

Figure 15–1. A stimulus filament near, but not yet touching, the tongue of a newborn infant (A) is brought into contact with the surface and a small indentation formed (B) as pressure is applied up to that amount required to bend the calibrated filament. A response that has as its minimal indication a displacement of the tongue tip from midline (C) may continue until the tongue has moved far to the ipsilateral side (D). The response is ipsilateral, being left going for left side stimulation and right going for right side stimulation. After maximal displacement, the tongue returns smoothly to its midline resting position without crossing to the other side.

perimentor rather than the subject controls the schedule of stimulation, repeated stimulation could be made in the absence of response and equal volumes of preferred and non-preferred fluids administered. The vastly improved specification of stimulus site would provide a stronger base for inferences from behavior to sensory mechanism. At a slightly more theoretical level it is to be noted that the use of elicited lateral tongue movements as an indicator removes the sensory cuing of differential response from the process of obtaining nutrient fluids.[20] An analogous separation of tasting or taste testing from drinking in the rat provides behavioral measures more clearly reflective of receptor activity.[2] By limiting ingestion to the minute samples required for sensory cuing, it is possible to achieve improved correspondence between the behavioral and electrophysiological measures of absolute thresholds[11] or of discrimination between suprathreshold stimuli.[16]

METHOD

To elicit the tongue movement response the infant is placed on his back and swaddled with his arms at his sides. The head is stabilized in a median orientation by one investigator who places one of his hands on each side of the infant's head. Another investigator opens the infant's mouth by gentle pressure on the chin. A 5 lambda sample of fluid is expelled from a micropipette* and formed into a drop that adheres to its tip. The drop is transferred to the tongue by touching its lower surface to a site near the tongue's tip and to the right or left of midline. The stimulus site, the relative size of the drop, and the transfer technique are illustrated in Figure 15–2. It is possible, in most circumstances, to maintain a stimulus schedule of one drop every 30 seconds. In the interval between stimulations, both investigators report whether or not they observed an ipsilateral displacement of the tongue tip from its original position at the midline. The sum of the number of positive reports is the subject's score. Interobserver reliability for the 66 infants of the present study is 92%.

EXPERIMENT 1

The first study employing the drop elicited lateral tongue motion response explores the discrimination of glucose solution from water. The subjects are 48 healthy, apparently normal, term newborns recruited from a local military hospital. Each subject is assigned to one of four stimulus concentration conditions by a random procedure constrained to yield groups balanced for sex and age (6–30 and 30–72 hours). The four stimulus conditions are water and glucose solutions of .28 M (5%), .55 M (10%) and 2.77 M (50%) concentration. Stimulation is in the form of 5 lambda drops of fluid individually applied to the right side of the tongue at the rate of one drop every 30 seconds. The response is a movement of the tongue tip toward the right side.

Initially each subject receives re-

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*Eppendorf Micro Pipet, distributed by Brinkmann Instrument, Inc., Westbury, New York.

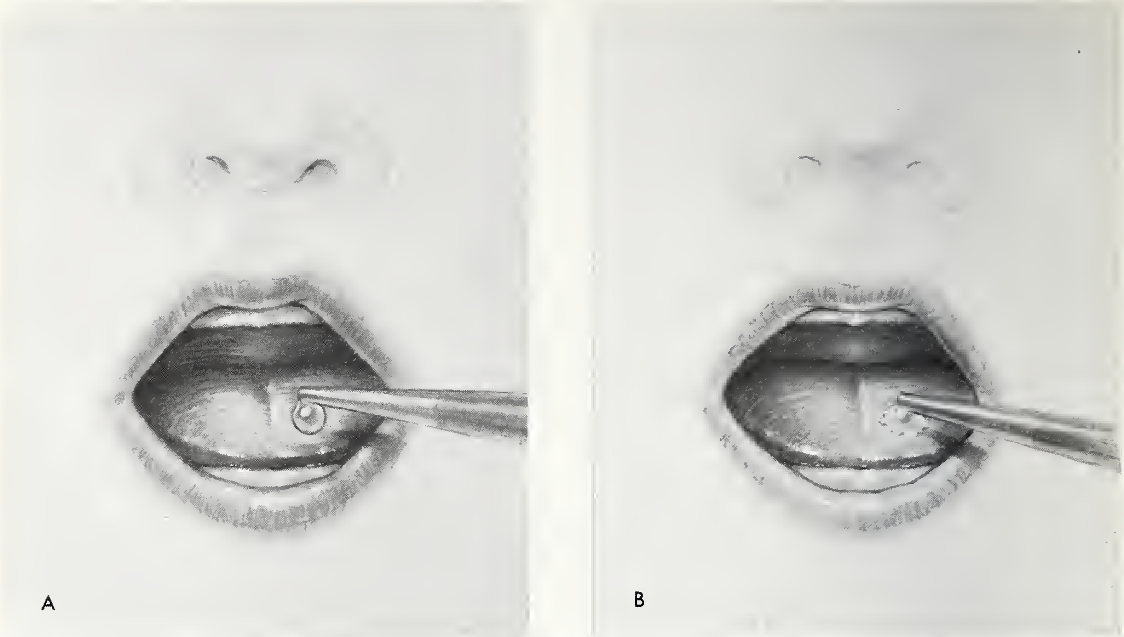

Figure 15–2. A measured microsample of fluid adhering to the tip of a pipette is held over the site of stimulation on the newborn's tongue (A). When the lower meniscus of the drop touches the tongue, the drop leaves the pipette to become a localized pool on the moist surface of the tongue (B). The response that follows this type of stimulation appears to be identical to that elicited by filament stimulation (see Figure 15–1).

peated stimulation with drops of water until responding is reduced to a uniform low level. This water adaptation or pretreatment phase continues until the infant reaches a criterion of 6 successive failures to respond. After reaching this criterion, each infant receives 12 more stimulations at the same site and on the same schedule as before. For some infants the test stimulus, like the adaptation stimulus, is water, others have a change in stimulus from water to one of three concentrations of glucose in water. A second trial using exactly the same procedures is carried out after a delay of at least 10 minutes. On trial 2 each infant is stimulated with drops of water to the previously used criterion of decreased response and then with 12 drops of the same test fluid that was used on trial 1.

Results

The groups tested with distilled water and glucose solutions of .28 M, .55 M and 2.77 M concentration achieve total scores of 55, 69, 85 and 166 respectively. Figure 15–3 displays these results separately for trials 1 and 2. Table 15–1 details an analysis of variance with 3 between subjects factors (age, sex and glucose concentration) and one repeated measures factor (trials). This analysis indicated a significant main effect for stimulus concentration (F=6.4371, df 3/32, $p<.002$). The main effects that would have indicated a difference in scores between the two age groups, the two sexes or the two trials all fail to reach significance (F=1.6945, F=0.1101, F = 1.6140, all with df 1/32, all ns.). there is a marginally significant interaction of

TABLE 15–1

SUMMARY OF ANALYSIS OF VARIANCE

Source	*df*	*MS*	*F*
Between Subjects			
Age (A)	1	27.093	1.6945
Sex (S)	1	1.760	.1101
Glucose Concentration (C)	3	102.927	6.4371***
A X S	1	27.094	1.6945
A X C	3	8.844	.5531
S X C	3	12.733	.7963
A X S X C	3	10.510	.6573
Subjects Within Groups	32	15.989	
Within Subjects			
Trials (T)	1	12.760	1.6140
A X T	1	.844	.1067
S X T	1	12.760	1.6140
C X T	3	19.177	2.4255*
A X S X T	1	3.760	.4756
A X C X T	3	2.372	.3000
S X C X T	3	5.844	.7391
A X S X C X T	3	8.733	1.1045
T X Subjects Within Groups	32	7.906	

***$p<.002$
*$p<.10$

stimulus concentrations with trials (F = 2.4256, df 3/32, $p<.09$).

The data for trial 1 are more orderly than those for trial 2. On trial 1, level of response is a monotonic rising function of concentration. The difference between scores for water and for .28 M glucose solution is small and nonsignificant while that between water and .54 M glucose is larger and significant (1.83 vs 4. 33, $t=2.06$, df=27, $p<.05$). On trial 2 the difference between water and each of the two lowest concentrations of glucose solution is non significant. The difference between water and 2.77 M glucose is significant on each trial. (trial 1; 1.83 vs 8.33, $t=5.0081$, df=22, $p<.001$: trial 2; 2.75 vs 5.50, $t=1.9928$, df=22, $p<.05$).

Discussion

If the elicitation of response as a function of the concentration of glucose in the test drops is to imply that there are glucose sensitive receptors on the tongue of the newborn, it is necessary to show that the responses have been elicited from tongue receptors. This assertion can be made on the basis of the observation that response can occur before the stimulus drop escapes from a restricted area on the tip of the tongue (see Figure 15–2). The fact that on at least some occasions the initial movement of a stimulus drop occurs as a consequence of the response elicited by that drop indicates that stimulation of additional receptors by movement or spreading of the drop from its initial site is not required for elicitation.

The present findings establish that glucose sensitive receptors on the tongue of the newborn are functional and invite comparison with other attempts to demonstrate glucose sensitivity in the newborn. The visual analysis of changes in sucking employed by Jensen[8] was sufficiently sensitive to demonstrate temperature thresholds and to show a graded response to salt concentration, but no differential response to 6% glucose solution as compared with milk was observed and the question of glucose sensitivity was not pursued. Dubignon and Campbell demonstrated a preferential ingestion of a standard nursery formula as compared to a 5% dextrose solution and suggested that the discrimination was based on taste.[4] Nisbett and Gurwitz observed differential ingestion of a commercial infant formula and a modified version in which the carbohydrates had been replaced by glucose.[18] Fortunately, considerably more appropriate comparison data are available from the studies Maller and Desor are report-

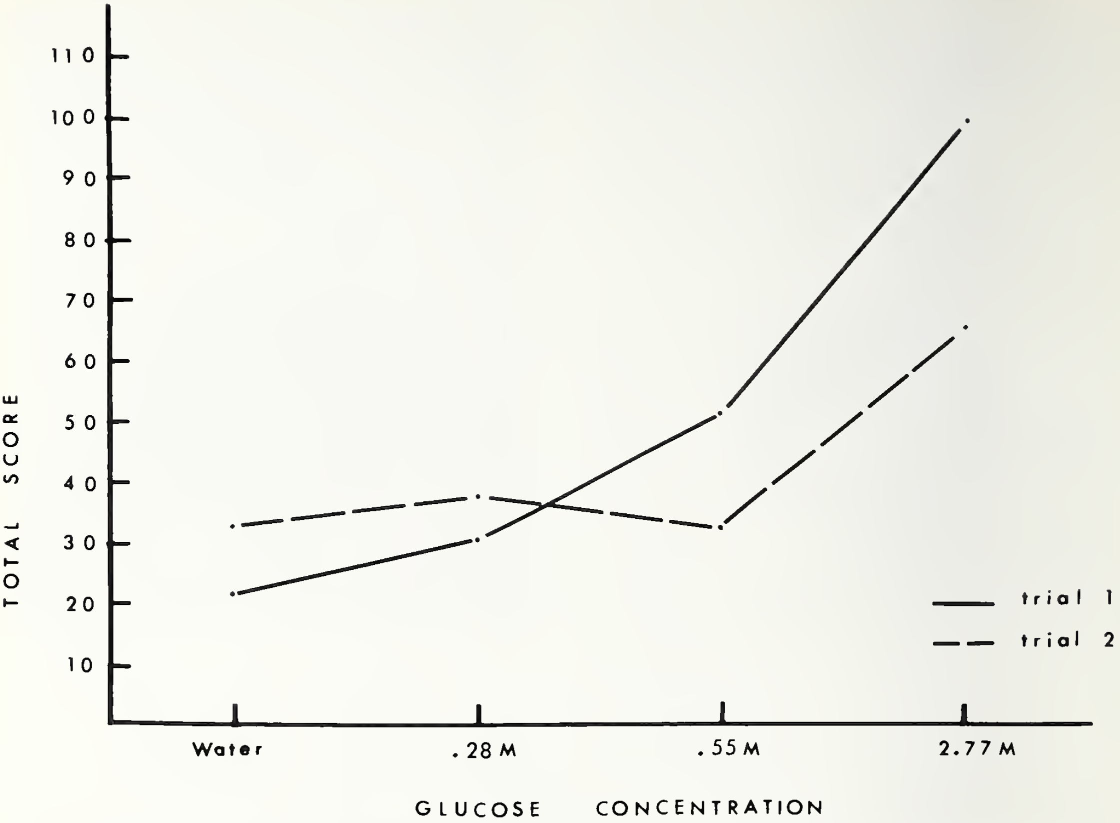

Figure 15–3. Total scores achieved by independent groups of 12 subjects on each of two trials as a function of the glucose concentration of 12 test drops applied after adaptation of the water response. Each of two observers assigned one score when an ipsilateral tongue movement was observed to follow stimulation with a drop of the test fluid.

ing in this Symposium.[14] In these studies ingestion of glucose solutions of various concentrations is compared with ingestion of water. Significant preferential ingestion is reported for concentrations lower than those required to demonstrate differential response when stimulation is by individual drops placed on the tongue. This is as one might expect on the bases of the inverse relation between area of stimulation and threshold for tastants in adults.[15] Infants in the .3 M glucose solution group of the ingestion study receive an average of approximately 12 ml (milliliters) of comparison fluid while infants studied by our method received only 120 lambda (microliters) of test fluid, a 100 fold difference in volume.

EXPERIMENT 2

In experiment 1 the test for discrimination of water from glucose solution takes place after repeated stimulation with water has reduced each subject's responding to a uniform low level. However, it is possible that this repeated water stimulation plays no role in determining the subsequent differential response to water and glucose solution and that discrimination of these fluids could be demonstrated without prior water stimulation. Measures of the initial level of response to water and the effects of repeated water stimulation are available from experiment 1. To obtain analogous measures of response to glucose solution a sample of 9 male and 9 female newborns was drawn from the same population and stimulated with 2.77 M glucose solution. The procedures are the same as those previously used except that all subjects receive initial stimulation with 2.77 M glucose solution followed by test stimulation with distilled water. Only one trial is administered.

RESULTS

The initial level of responding to water on trial 1 of experiment 1 is compared to the initial level of responding to glucose solution on the single trial of experiment 2. Scores for the first 6 stimulations with water are not significantly different from scores for the first 6 stimulations with 2.77 M glucose solution (6.11 vs 5.52, t=.7451, df=64, ns.). Analysis of the decrement with repeated stimulation also fails to give evidence of differential response to water and glucose solution. Neither the number of stimulations required to reach the criterion of 6 successive failures to respond nor the score obtained during this procedure differentiates water from glucose solution (number of stimulations; 10.6 vs 10.5, t=.7239, df=64, ns.; scores; 8.33 vs 9.5, t=.1984, df=64, ns.).

The group in experiment 1 that receives water as a test stimulus demonstrates the effects of repeated water stimulation on subsequent response to water. Significantly fewer responses are elicited by water during the 6 stimulations immediately after reaching the criterion than by the water during the first 6 stimulations of the trial. (6.00 vs 1.50, t=4.7395, df=11, p.<.001). However, this level of response to water after repeated water stimulation is not significantly different from the response to water after repeated glucose solution stimulation to the same criterion (1.5 vs 1.5 for 1st 6 stimuli; 1.8333 vs 2.4444, t=.7792, df=28, ns. for all 12 stimuli). Water and glucose solution are equally effective in reducing the response to subsequent water stimulation.

The stimulus change that precedes testing is equal in magnitude but opposite in direction for the experiment 1 group that has repeated water stimulation followed by a test with glucose solution, and the experiment 2 infants initially stimulated with glucose solution and subsequently tested with water. Test scores following an alteration of the stimulus by the addition of glucose are significantly greater than the scores on tests following the deletion of glucose from the stimulus fluid (8.33 vs 2.44, t=2.4950, df=28, p<.01).

CONCLUSIONS

The findings of experiment 1 imply peripheral sensory mechanisms capable of resolving a difference between glucose solution and water. The additional comparisons provided by experiment 2 contribute by placing constraints on the choice of theoretical models for specifying the sensitivity of these receptor mechanisms and the nature of the interactions between them.

Repeated stimulation reduces responding to a low level. In experiment 1, continuation of water stimulation yields a low level of response that constrasts sharply with the increment in response associated with a stimulus change from water to glucose solution. These data suggest that an increment in response is occasioned by an increase in glucose concentration. However, they are equally consistent with an account in which the increment is determined by a simple change in concentration, regardless of its direction. Such a response to stimulus change *per se* has been used to implicate a central mechanism in the original suppression of response by repeated elicitation.[7,9] However, experiment 2 indicates that the response to a change in glucose concentration is dependent on the direction of the change. An increment, but not a decrement, in glucose concentration leads to increased responding during subsequent testing. Therefore, an account in terms of peripheral mechanisms of sensory adaptation is adequate. It is unnecessary to invoke a central mechanism of habituation to account for the critically important changes that occur as a function of repeated elicitation.

The sensory mechanisms responsible for elicitation of the lateral tongue response must provide both for the equivalence of response to glucose solution and water prior to water adaptation and for the differential response to these fluids after water adaptation. One highly unlikely, but logically possible, account posits receptors specific to each fluid in its totality, a water receptor and a glucose solution receptor. Before adaptation both receptors are equally effective. Adaptation of the water receptors spares the equally effective glucose receptors which are then available for the post adaptation test. This is the type of model for receptor sensitivity that Bridger [3] developed in his studies of auditory discrimination in the newborn. However, this type of account can be ruled out, in the present case, because it is inconsistent with the finding that repeated stimulation with glucose solution has an effect on subsequent response to water. The sensory mechanisms stimulated by water and glucose solution cannot be entirely separate and unique.

The differential response to glucose solutions as a function of their glucose concentration demonstrated in experiment 1 may result from the operation of a receptor mechanism sensitive not to glucose solutions *per se* but to the glucose component of such solutions. A chemosensitive receptor mechanism responding either specifically to glucose, or more generally to a variety of sugars or sweet substances, would not be affected by the repeated water stimulation of the adaptation procedure, and would therefore be available to provide post adaptation response to glucose solutions. The fact that repeated stimulation with glucose solution produces a decrement in subsequent response to water suggests that glucose solutions stimulate not only a glucose sensitive mechanism but also the receptor mechanism which triggers response to water. By this account, the water sensitive mechanism is stimulated either by water or glucose solution and is responsible for the initially equivalent response to these fluids. After adaptation of the water sensitive receptors, the glucose solution elicits response by virtue of the fact that it can stimulate not only this mechanism but also the glucose sensitive chemoreceptors.

The receptor mechanism sensitive not

only to water but also to solutions of glucose in water might be described as a water receptor. However, this description of the mechanism's sensitivity does not adequately specify the common property or attribute of the two fluids that elicits response. Since the fluids are applied at room temperature there is a temperature differential between the stimulus and the surface of the tongue. Thermal receptors, therefore, provide a possible mechanism of response to shared properties. We have neither manipulated temperature as a stimulus parameter, nor attempted to match the temperature of our stimulus to that of the moist tongue in air. Previous studies of response to orally presented fluids differ enough from the present circumstance to leave open the question of whether a temperature differential of this magnitude would be adequate to elicit response.[8,19]

Since all of the drops provide contact or "fluid touch" stimulation, mechanoreceptors could form the basis for nonspecific response to drops. One might even suggest that fluid touch is merely an alternative technique for stimulating the same receptor mechanisms activated by filament stimulation. By this account fluid touch stimulation, equally provided by either water or glucose solution, underlies the equivalence of preadaptation responding. Water stimulation adapts the receptors for fluid touch but since glucose stimulates not only fluid touch but also glucose receptors, post adaptation tests show differential response. Repeated stimulation with glucose solution adapts both the glucose sensitive mechanism and the fluid touch receptor and thus has an effect equal to that of water adaptation on subsequent water tests.

An analysis in terms of the response of fluid touch sensitive and glucose sensitive receptor mechanisms serves as a reminder that the vehicles or solvents that necessarily accompany tastants to the tongue may themselves have stimulating properties. Failure to take account of these properties may underlie some of the divergence among results obtained in studies of newborn taste. Those studies where glucose or sugar is one, but not the only, constitutent that differentiates two test fluids[4,6,12] have shown either no differences between fluids or a preference for the comparison fluid. Studies where the difference between the fluids have been limited to a single constituent and thus do not confound differences due to sugar with differences due to other taste or non taste properties have uniformly shown preferential ingestion of the fluid containing sugar.[10,14,17,18]

SUMMARY

The elicited lateral tongue movement response has been shown to be a viable indicator response for the demonstration of the sensitivity of the newborn's tongue to glucose. A discrimination of water from glucose solution and an orderly relation of response to glucose concentration has been shown on the basis of equal micro volumes of comparison fluid. The adaptation paradigm in conjunction with the directional tongue movement response is an extension of techniques successfully employed in the psychophysics of infant vision, audition and olfaction, and provides a unique opportunity to separate the peripheral mechanisms of chemo-, mechano- and thermo-sensitivity that underlie the infant's sense of "taste."

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DISCUSSION

SUMINO: Is this response peculiar to the newborn baby?

WEIFFENBACH: Our primary studies of this response have been with term born babies during the first three days of life. With premature infants, we have followed it serially through the 1st month and a half or two months of life. There are a few normal infants that we have seen sporadically through the 1st year of life. The elicited ipsilateral tongue response appears to be absent in adult humans although we have seen it in a few adult subjects with other peculiarities of oral behavior or of medical history. Our attempts to elicit lateral tongue movements from infant mammals of other species have been unsuccesful, but I attribute this to our failure in setting up an appropriate situation.

SUMINO: I have an interesting finding in the cat which is related to your work. I electrically stimulated branches of the lingual nerve and recorded reflex discharges in branches of the hypoglossal nerve. Thus responses were recorded in the particular branch of the hypoglossal nerve which innervated the tongue muscles adjacent to the receptive field of the stimulated branch of the lingual nerve.

WEIFFENBACH: That is very exciting. As you might have imagined, our attempts to elicit the response from other species was for the purpose of finding a suitable preparation for a neurophysiological study of this behavior.

SUMINO: Can you tell us about the latency of this response of transverse motion in the tongue following touch stimulation?

WEIFFENBACH: We have not yet worked out a satisfactory procedure for measuring latency from the video recordings though this is a project that we are working on. With cinema recordings, on the other hand, it is a relatively simple matter to count the number of frames between the first contact of the stimulus with the tongue and beginning of the lateral deflection and thus calculate latency. Although our sample is small, it would appear that 50% of the response to filament stimulation have latencies between 250 msec. and 500 msec. With elicitation from fluid drops, on the other hand, the analogous range appears to be approximately 150 msec. to 300 msec. I am very anxious that we should reach the point where we can measure latency from the video recordings since under those circumstances we would be able to collect enough data for the comparison of latencies to water stimulation with latencies to tastant stimulation. Thus potentially differentiating what I believe is the response to the tactual component of drop elicitations from a, perhaps, different receptor mechanism having to do with taste.

MUNGER: I wonder what type of sensory receptors are stimulated by water? Mechanoreceptors, thermoreceptors and taste receptors are all present and could be activated by water placed on the tongue.

WEIFFENBACH: I really don't know. Faced with this situation one can adopt the viewpoint that the drop stimulation probably stimulates all the receptors; that is, the mechanoreceptors, the thermoreceptors, and the taste receptors. In this view the adaptation procedure reduces the influence of one type of receptor *vis a vis* the others. The stimuli are all delivered at room temperature and are the same size. Therefore, by repeatedly presenting water and then shifting to another fluid which is much like it except for the addition of a tastant, we are assuming that we have adapted the receptors sensitive to that particular temperature and that particular mechanical presentation and elicit a response which is a function of the *difference* in taste between the fluids.

If, on the other hand, your question is directed to the matter of whether water has a taste or not, the answer is even more difficult. If one wished to draw an anology between the *repeated* stimulation applied to infants and the *continuous* stimulation used in demonstration of "water taste" in adults[1] the failure to get a response to water after glucose adaptation could be taken as evidence against the water taste phenomena in neonates. This would suggest that the tongue responses we ordinarily see to water are primarily a function of the mechano- or thermo-receptors.

BOSMA: The variation in receptors is compounded by differences in the afferent routes. Mechanical touch is presumably a trigeminal afferent and taste is carried by chorda tympani to the facial nerve trunk. But I believe the afferent route of fluid touch is still in question.

BRADLEY: I am concerned that your taste stimulus may not remain at either the apex or the lateral borders of the tongue. Since the tongue is covered by an aqueous layer, rapid diffusion of the taste stimulus in this layer will occur. Calculations can be made of the diffusion time and the change of concentration with respect to time.[1] How have you tried to control for this possibility? For example, perhaps you could use a drop of dye to ascertain how localized the drop remains with time.

WEIFFENBACH: Diffusion of the fluid across the surface of the tongue, which would bear on the question of stimulus site localization, is many times slower than the diffusion through the aqueous layer of the receptors that Beidler calculates as a part of the delay between stimulus presentation and response of first order neuron. In fact, frame by frame viewing of color cinema made during stimulation with formula often shows that the area of tongue covered by the drop is constant during most of the time between when it is placed on the tongue and when its contours are disrupted by the movements of response.

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[1] Beidler, L.M.: Taste receptor stimulation. In Butler, J.A.V., Huxley, H.E., and Zirkler, R.E. (Eds.) *Progress in Biophysics and Biophysical Chemistry*, New York, Pergamon, 1962, pp. 107–151.

Chapter 16

THE LATERAL TONGUE REFLEX IN LOW BIRTH WEIGHT INFANTS

BRADLEY T. THACH AND JAMES M. WEIFFENBACH

A lateral movement of the tongue toward the side of stimulation occurs as part of the rooting response in the newborn human infant[3,4,5,20] and has been elicited by touch stimulation of the lip of fetal sheep.[6] A light touch stimulus delivered to the right or left side of the infant's tongue tip also elicits ipsilateral tongue movement,[21,22] Our initial studies in term newborn infants indicate that this reflex can serve as an indicator response for studies of oral touch sensitivity in the newborn.[21] This reflex has properties that are required for such testing. The frequency of reflex elicitation is positively correlated with stimulus intensity and is relatively independent of the infant's state of arousal. The movement is directed toward the stimulus, thereby reducing the problems involved in distinguishing spontaneous or adventitious movements from elicited movements. The response is remarkably consistent: it has been elicited in every infant that we have tested.

However, in order for the reflex to have broad applicability for sensory testing in infants, other questions need to be answered. In term infants, there appears to be a decrease in responsiveness over the first three days of life.[21] In view of this, it is important to investigate long term changes in responsiveness. Another question that needs to be answered is that of the relation between responsiveness and gestational age.

Our studies of low birth weight infants have been directed to these questions. Most of these infants are premature ($<$ 37 weeks gestation). They may remain hospitalized a number of weeks after birth and therefore offer opportunity for long term observation in a controlled environment. Furthermore, these studies are of particular interest since so little information is available on the quantitative assessment of sensory function in short gestation infants. In this Chapter we report our findings on the first 24 infants tested in a study that is still in progress.

Acknowledgment: The authors are indebted to Barbara Thach and Martha Bosma for assistance in testing subjects, to Dr. Thomas Reichelderfer and the staff of the District of Columbia General Hospital Newborn Intensive Care Nursery in making this study possible and to Dr. James Bosma for contributions to the preparation of this manuscript. This research was carried out and the present report prepared during the second author's tenure of a National Institute of Dental Research Senior Staff Fellowship.

METHODS

Only infants who are judged by nursery staff physicians to be generally healthy are enrolled in the study. Many of the infants under 35 weeks estimated gestational age, however, are receiving intravascular fluids and oxygen therapy at the time they are first seen. Preference for enrollment in the study is given to the smallest infants available on the day of our weekly visit to the nursery*. All infants are born to Negro mothers and came from the same large urban hospital nursery. Infants are initially tested by our routine of examination in the first four days of life (mean age ± S.D. = 36.3 ± 24 hours). Each infant is retested at two week intervals for the remainder of his hospital stay. A shortened form of the testing procedure developed for term infants[21,22] is employed with the modification that most of the infants are immobilized by the hands of one of the investigators rather than being swaddled in a blanket. The infants are tested while lying supine without being removed from their incubators or warming beds.

Test stimuli are a series of 8 individual nylon filaments of graded diameter**. They are designed to exert a standard force when pressed against a surface. The heaviest and lightest gauge filaments in the series yield a force of 3.60 and .06 grams respectively.[18] Each of the stimuli is applied once to each side of the tongue in predetermined random sequence. Then, without temporal break, a second series of 16 stimulations is applied, using a different random order. The interstimulus interval is 30 seconds.

Since menstrual histories obtainable from the mothers are not considered to be uniformly reliable, gestational age is estimated. After testing, each infant is examined for specific physical and neurological criteria of maturity. Gestational age for each infant is estimated on the basis of the degree of maturation of 9 physical and 6 neurological signs, by using Lubchenko's gestation assessment chart.[15]†. Estimated gestational age for the 24 infants ranged from 31 to 39 weeks (Fig. 16–1).

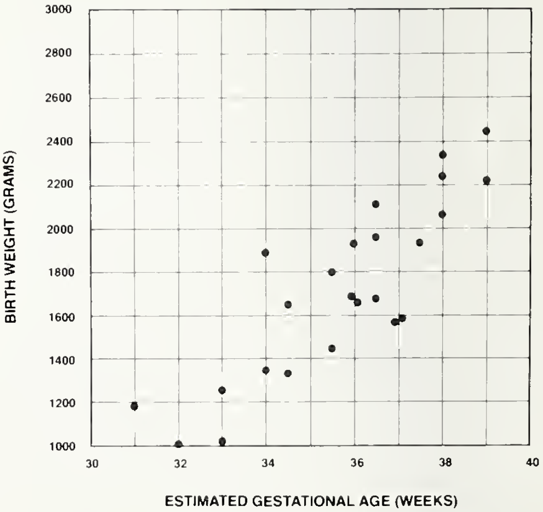

Figure 16–1. Birth weight as a function of estimated gestational age for the 24 infants of this study.

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*All infants fell well below the 50th percentile and 16 slightly below the 10th percentile for weight based on standard intrauterine growth curves (Usher *et al*[19]) and our estimate of gestational age. From the standpoint of birth weight, the infants are a fairly homogeneous population (Fig. 16–1) even though over half of them can be considered "small for dates".

**Semmes-Weinstein esthesiometers were obtained from Research Media Co., Syosset, New York.

† The physical signs were: breast tissue, nipples, sole creases, ear cartilage, ear form, genitalia, lanugo, skin texture and resting posture. The neurological signs were: extremity recoil, scarf maneuver, neck extensors, neck flexors, grasp reflex and heel to ear maneuver. In 13 infants, another standardized method of estimating gestational age by physical examination (Dubowitz *et al*)[8] was used in addition to our routine examination.

Observations

We found our examination readily adaptable to the study of premature infants. As a rule, premature infants have less mandibular tone and opening the mouth requires less pressure on the chin than in mature infants. The occasional lifting of tongue tip to palate which can interfere with testing in the mature infant is seldom seen in the premature.

In general, the lateral tongue reflex in the premature infant was identical to that elicited by touch or fluid drop stimulation in older infants. The reflex was elicited in all infants at all testing sessions. In 48 of 54 separate test sessions, responses were seen to at least 40% of all stimuli. As in term born infants, elicitation of this reflex is a positive function of stimulus intensity. This is reflected in the stimulus response curve (Fig. 16–2) in which the averaged response frequencies of infants tested at the initial test session are plotted against stimulus intensity. The weakest intensity stimulus produced less than 16% responses and the strongest produced greater than 95% responses. The stimulus response curve is S-shaped. This configuration appears to reflect the presence of a threshold. The probe that delivers a stimulus intensity of 1.50 grams gives approximately 50% responses, with stimulus intensities on either side, in the serial order, giving 16% and 70% responses.

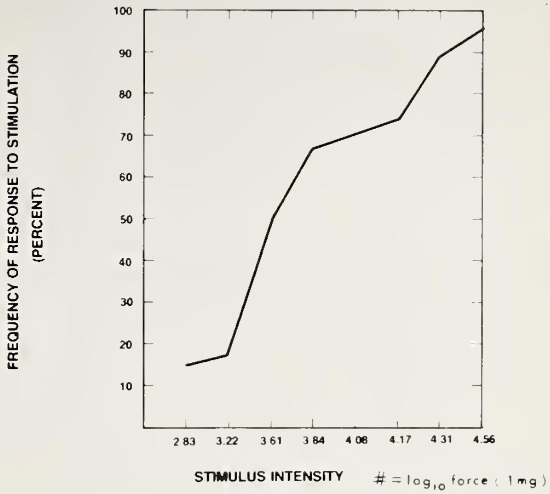

Figure 16–2. Frequency of elicitation of lateral tongue responses as a function of tactile stimulus intensity. Averaged scores of 24 infants each tested with a total of 32 stimulations in the first 4 days of life.

The group as a whole decreased in frequency of response (percent response to all stimuli) at 2 weeks compared to the initial neonatal response frequency (48.6% *vs.* 56.6%, N=18, t=3.41††, $p < .005$). However, for those infants followed four weeks and longer, this decrement does not continue (Fig. 16–3). At four weeks, the average frequency of response is increased over that at two weeks (60.9% *vs.* 50.7%, N<7, $t < 5.39$, $p < .005$) and is not significantly different from the frequency in the neonatal period (59.3% *vs.* 60.9%, N= 7, t = 1.0, N.S.).

In spite of individual variations in responsiveness there is a high degree of correlation in scores obtained with repeat tests at two week intervals (Fig. 16–4). The neonatal response frequency is correlated with the frequency at two weeks (N=18, r= .71, $p < .001$) and the frequency at two weeks is correlated

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There was a significant degree of correlation between the two (N = 13, r= .94, $p < .001$), although estimates by Dubowitz's method averaged 1.5 weeks less than estimates by our method. The gestational age derived by averaging estimates based on the matching of birth weight, head circumference, and crown heel length to the 50th percentile of intrauterine growth curves[19] also is correlated with our estimation of gestational age (N=24, r=.88, $p < .001$).

†† Computation of values of t and r are on the basis of number of observed responses. Results are reported as percent response, which is the number of observed responses divided by the number of stimulations (16 per trial, 32 per test session).

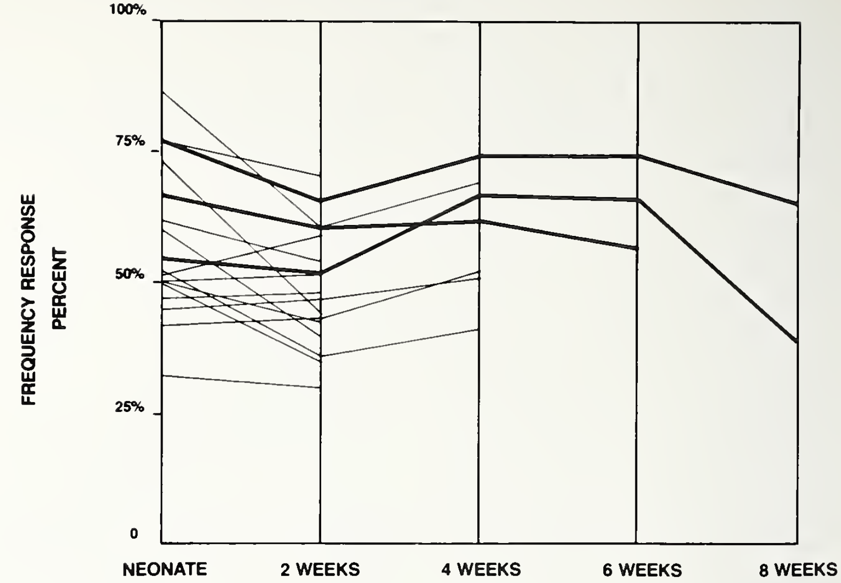

Figure 16–3. Frequency of elicitation of lateral tongue responses for 18 low birth weight infants tested at 2 week intervals from birth until time of hospital discharge.

with that at four weeks (N=7, $r = .90$, $p < .001$). In other words, the responsiveness of an individual infant relative to the other infants in the group tends to be constant, although the average responsiveness of the population as a whole varies from week to week.

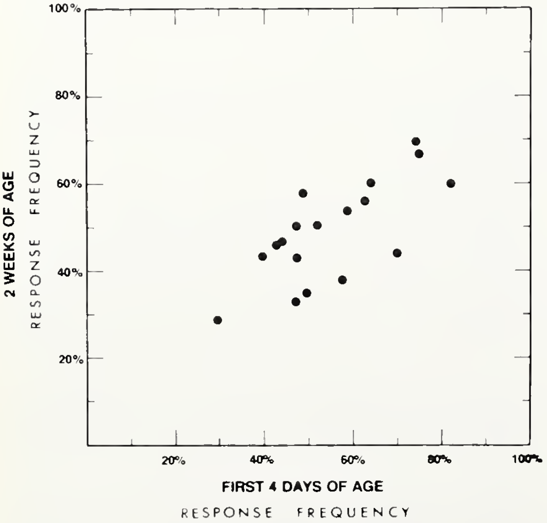

Figure 16–4. The correlation of the frequency of response elicitation at 2 weeks of age with that in the newborn period for 18 infants.

In the neonatal period, the response frequency of individual infants is variable. However, some of the shortest gestation infants were the most responsive (Fig. 16–5). The scattergraph of response

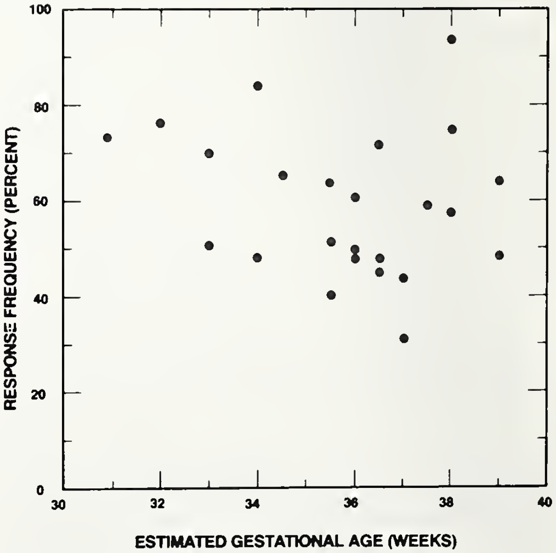

Figure 16–5. The relation of frequency of lateral tongue response elicitation to estimated gestational age for 24 infants tested in the first 4 days of life.

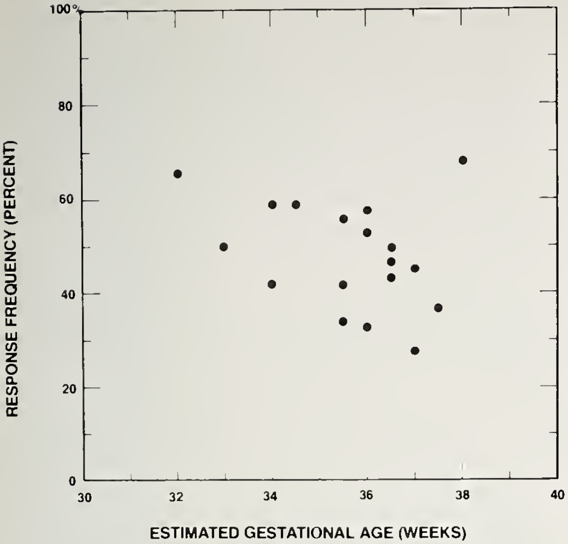

Figure 16–6. The relation of frequency of response elicitation to estimated gestational age for 18 infants retested at 2 weeks of age.

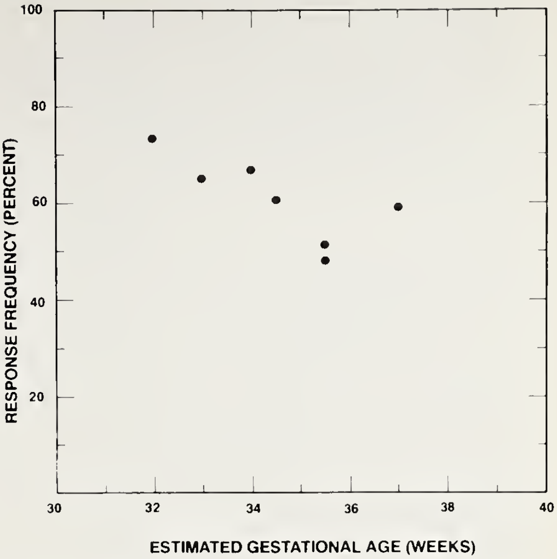

Figure 16–7. The relation of frequency of response elicitation to estimated gestational age for 7 infants retested at 4 weeks of age.

frequency at two weeks appears more orderly than in the neonatal period (Fig. 16–6). This pattern persists at four weeks of age, where there is a significant negative correlation between estimated gestational age and percent response ($N= 7$, $r=.76$, $p < .05$) (Fig. 16–7).

There is a significant degree of correlation between the first stimulation trial and the repetition ($N=24, r=.68, p<.001$) and the response frequencies in each trial were not significantly different (60.5% *vs.* 58.1%, $N=24$, $t=1.0$, N.S.). The frequency of response to right sided stimulation was not significantly different from that to left sided stimulation (58.2% *vs.* 61.2%, $N=24$, $t=1.13$, N.S.). The sexes did not differ with respect to frequency of response elicitation (61.6% *vs.* 54.9%, $N=24$, $t=1.03$, N.S.).

SUMMARY

It is interesting to compare the results of this study with those from our previous study of term born infants.[21] The overall similarity of the tongue reflex in the premature low birth weight infant compared with the term born infant was surprising to us since many infantile reflexes including rooting, sucking, and turning to light are more difficult to demonstrate in the premature infant.[1,2,11,17] The stimulus response curves[21] (for the same range of stimuli) are similar for both groups. In the first four days after birth, the overall frequency of response (60–80%)) is also similar for both groups. That the shorter gestation infants in the low birth weight population appear to be more responsive than the more mature infants of this population is difficult to account for since the population when considered as a whole is so similar to full term infants. The previously studied term born infants and the low birth weight infants are from different socio-economic and racial backgrounds, so perhaps the comparison shouldn't be carried too far.

The decrement in response frequency seen at two weeks of age, in premature infants, is followed by a return in following weeks to higher levels of response. This has importance with respect to utilization of the reflex in sensory evaluations, since it suggests that the reflex has a degree of stability and can be expected to occur in infants well beyond the newborn period.

How can we explain these changes in response frequency that occur over weeks or days? In order for a response to occur, a reflex arc must be completed. A deficit or inhibition at any point will result in failure of response. Changes in response frequency might be expected to result from a change in (1) the mechanical properties of tissue overlying the receptor, (2) receptor threshold (3) the state of receptor maturation, (4) the density of receptors within the tissue and (5) the central connections of the reflex. Most of these factors have already been cited by other investigators to account for differences in tactile sensitivity as a function of age[7,9,12] area of the body[10,18] sex[7,9] and skin temperature.[14,16] Barcroft and Barron's work with fetal sheep[6] and Humphrey and Hooker's[13] observations in human fetuses provides evidence that certain touch elicited fetal reflexes are altered in form or may disappear altogether with maturation. This is presumed to be a result of inhibition of primitive brain stem functions by other areas of the maturing brain. These observations would seem to be significant in the interpretation of our findings.

During the first postnatal month, the elicitability of the tongue reflex is not a linear function of postnatal age. It is initially high in the newborn, decreases at two weeks and increases to the neonatal level at four weeks. It seems that no single hypothesis based on maturational changes in central reflex inhibition or in peripheral receptors can explain these findings. It is well to note, however, that such changes are not large. The overall impression we have of this reflex is that of relative stability. With an adequate stimulus the tongue reflex is, in large part, "obligatory".

So far, we have been referring to populations and pooled scores. What is the significance of a determination in an individual infant? The high degree of correlation between the response frequencies assessed at two week intervals indicates that response frequency is the function of relatively stable, intrinsic properties of the individual. It suggests that previous sensory experiences or other short term environmental variables may be less significant in determining response frequency than we had previously expected.

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DISCUSSION

BRACKBILL: For subsequent studies in which you need to use gestational age estimate, I suggest that you try the Dubowitz, *et al*,[1] scale for estimating gestational age. I think you would probably find that to be more reliable and more generalizable to the whole population of infants.

For example, the single measure of head circumference is not equally applicable to caesarean and vaginal deliveries. The Dubowitz, *et al*.[1,2] scale has two subscales in it, each with about ten items, one called external signs and the other neurological signs. The neurological signs are affected by medication but the external signs are not. They are relatively stable and therefore a good estimate of gestational age.

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[1] Dubowitz, L.M.S., Dubowitz, V., & Goldberg, D. Clinical assessment of gestational age in the newborn infant, *J. Pediat.*, 1970, 77, 1–10.

[2] Bronshtein, A.I., & Petrova, E.P. Issledovanie zvukovogo analizatora novorazhdennykh i detei rannego grudnogo vozrasta. (An investigation of the auditory analyzer in neonates and young infants.) *Zhurnal vysshei nervnoi Deiatelnosti*, 1952, 2, 333–343. Reprinted in Y. Brackbill & G. Thompson (Eds.), *Behavior in infancy and early childhood: A book of readings*. New York: Free Press, 1967.

And it is very easy to administer this scale. In fact, the original article that carried a description of the scale has assessments of reliability for both trained and untrained observers.

THACH: I chose Lubchenko's system[1] for estimating gestation because I have had more experience with it. We have, in fact, tried the Dubowitz system on half our infants. As far as the actual signs are concerned, it is similar to Lubchenko's. All but one of the external signs in the Dubowitz examination are also in Lubchenko's. By considering neurological signs as well as external signs, Dubowitz has reduced the error of the estimate based on external signs alone (± 2.4 weeks at 95% confidence),[2] by half a week. However, I found the Dubowitz system to be less applicable to our population of infants than the system we used. In attempting to be objective it has sacrificed flexibility. In order to estimate gestation using the Dubowitz regression curve, one must score all items of the examination. For instance, if you wish to estimate gestation in an infant who is known to have received medication prenatally, you cannot use external criteria and ignore the altered neurological signs without a significant modification of the Dubowitz system. In Lubchenko's system you are not bound to use any data which you feel may be unreliable or which is unobtainable. From a practical standpoint we were frequently unable to perform the ventral suspension test required by the Dubowitz examination because of hindrance from intravenous tubing. The Dubowitz method was developed in caucasian infants, as far as I know, and from my own experience, two items on the examination—skin color and opacity—will either have to be rescored or omitted before the examination is applicable to a population of black infants such as ours. In using Lubchenko's method, we felt justified in discounting these signs.

MUNGER: What exactly is the change in threshold? Would not a threshold be more meaningful than percent response.

THACH: We have been guarded in our use of the term "threshold". For a given measurement of sensitivity, threshold is operationally defined. Touch thresholds in adults are usually determined by presenting stimuli ordered according to intensity in ascending or descending order. The first or last stimulus to which the patient responds is taken as threshold. One might also present stimuli in random order, as we have done, and by interpolating from a curve of stimulus intensity *vs.* response frequency obtain a value for an intensity predicted to give 50% response. However, to justify interpolation one needs a relatively smooth curve. In an individual infant there were only 4 trials of each stimulus intensity, and we frequently do not have a nice S-shaped response frequency curve. Nevertheless, for each infant in the series, response frequency tends to be less to weaker stimuli and more to stronger stimuli. In the more responsive infant, we are likely to see a greater number of responses to intermediate intensity stimuli than in a less responsive infant. The more responsive infant, therefore, has a greater total of responses. The area of maximal slope in his response frequency curve will be moved to the left. The "threshold" value predicted to give 50% responses, interpolated from this curve, would be reduced. In a general sense, therefore, we feel that total or "percent" response is inversely proportional to receptor threshold. In the response frequency curve for pooled data of all infants, the point of greatest slope in the S-shaped curve seems to indicate a receptor threshold, but, this curve is a generalization for the population as a whole. For the population, the intensity giving 50% response was close to .4082 gm.

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[1] Lubchenko, L.O.: Assessment of gestational age and development at birth. *Pediat. Clin. N. Amer.*, 1970, 17:125–131.

[2] Farr, V.: Estimation of gestational age by neurological assessment in the first week of life.*Arch. Dis. Child.*, 1968, 43:353–.

MUNGER: As you get to a smaller and smaller stimulus the probability of hitting a receptor becomes less. Some receptors have exceedingly small receptive fields.

THACH: The size of the receptive field is no doubt important, and this could possibly change with fetal maturation. Another factor that we have speculated on is the concentration or density of receptors in the integument. In the last 10 weeks of gestation, the linear dimensions of the tongue nearly double. If no further ingrowth of nerves occurs during this time, then the density of endings might be less in the term fetus.

KAWAMURA: What kinds of sensory functions of the tongue develop first? Have you ever used a different kind of stimulus in your experiments? And what kind of stimulus is the first to elicit a mature response?

THACH: In premature infants our systematic studies have been restricted to touch-pressure sensitivity using Semmes–Weinstein filaments. We have, however, elicited the lateral tongue reflex with 5 μl drops of water in one or two premature infants and these appeared essentially the same as drop elicited responses in term infants. I don't know whether or not the drop stimulates touch receptors—no deformation of the mucosa can be observed when it is applied. Furthermore, we have no evidence in the infant indicating whether or not taste receptors are stimulated with distilled water drops.

Temperature seems to be an important factor. In our test situation the fluid stimulus is at room temperature. In adult subjects a 5 μl drop of water placed on the tongue is usually reported as "cool". When drops are warmed to near body temperature the intensity of stimulation is subjectively much less. If a paramedian site 3–4 cm behind the tongue tip is stimulated, warming the fluid drop has resulted in a decrease in recognition from 80% to 40% in adult subjects. Therefore, I feel that the thermal component of the drop stimulus is highly significant.

Our use of the term "fluid touch" may be misleading. We have also used a small blast of air to elicit this response in term infants. In this case the stimulus is mechanical, since the mucosa is slightly deformed, and is probably also thermal.

As to which tongue sensory functions develop first, that is an important question, and I know of no studies evaluating the different modalities in the young fetus.

Chapter 17

THE GUSTOFACIAL RESPONSE: OBSERVATION ON NORMAL AND ANENCEPHALIC NEWBORN INFANTS

JACOB E. STEINER

I shall describe and discuss the phenomenon which has been named the "gustofacial reflex".[34] This is a well-differentiated motor reaction of the facial muscles to taste stimuli. It is a rigidly fixed feature of innate character, and it is controlled by neural structures of the brainstem. This reaction is interpreted as a stimulus-dependent human nonverbal communication pattern.

Physical and chemical events originating from either the external or internal environment of a living organism become sensory stimuli when the organism is equipped with appropriate sensory receptors. These receptors are able to transduce the energetic event and encode it into the language of neural signals. Signals coming from the peripheral receptors become meaningful messages for the organism when the nervous system is able to decode them and to utilize the sensory input as relevant information. Living organisms can respond to peripheral sensory stimulus by a broad spectrum of reactions. These can involve either the organism as a whole, or be related to particular organ systems. Sensory stimuli may be answered by responses which are complex, involving cognitive and psychic processes.

Communication between living organisms is based on neural functions, which may be analyzed in their basic elements as centrifugal (efferent) reactions to centripetal (afferent) neural input. Since one of the most typical characteristics of communication signals is their transient nature it is therefore necessary to analyze them in their temporal arrangement. In such an analysis, diverse technical aids must be used to transpose temporal intervals and sequences of events into spatial terms. This is done when the experimentor records sounds on a magnetic tape or takes motion pictures. These aids make possible precise recording of the processes and ensure reliable and reproducible documentation.

Another important property characteristic of communication signals is the fixed pattern of the elementary signal as agreed between emitter and receiver. Human communication is built upon both verbal and nonverbal signal systems. The nonverbal system includes mimicking, gestures, and postures. The term verbal communication is used for both verbal communication *sensu stricte* and for other signals involving vocalization. The field of study dealing with all types of human communication has recently been named "anthroposemiotics". The verbal and vocalizing repertoire of human communication is impressively rich and potent in its use, as compared with the nonverbal repertoire. It is therefore easy to understand why the main effort in anthroposemiotic research is directed more toward the analysis of vocal and verbal signals, and less toward the nonverbal.

A century ago, Charles Darwin in his classic, "The Expression of the Emotions in Man and Animals",[5] employed the evolutionary approach to understanding of nonverbal communication signals. The findings of "zoosemiotics" (The study of the expressive and communicative signaling in animals) has a large impact on anthroposemiotics. According to Sebeok,[28] there is little doubt today about the importance of observations on animals for the better understanding of human nonverbal signals and the rules of their activation.

The nonverbal communication repertoire is built of elements which are common to man and other mammals and even to lower vertebrates. Head and body postures and stereotypes of leg posture and movements, as well as facial movements, are basic elements of the nonverbal catalog.

The contribution of modern sensory physiology to the analysis of the nonverbal signal system becomes relevant when a rigidly fixed communication signal can be elicited by a well-defined stimulation of one sensory modality and the neural pathway responsible for the particular sensory-induced reaction can be elucidated by laboratory methods. When one particular reaction of one or more organ systems can be repetitively elicited by precise stimuli and the induced reactions have rigidly consistent features, then a stimulus dependent communication signal is demonstrated.

We carried out several studies in our laboratory dealing with the functional state of the sense of taste in human beings.[12,30,31,33] By a simple psychophysiological test-procedure we tested adults, some under normal physiological conditions, others under paraphysiological or pathological conditions. Stimulus delivery was in accordance with the "greco-latin square test design", as adapated by our laboratory.[12] While we carried out these studies, we became aware of a striking phenomenon: suprathreshold sweet, sour and bitter stimuli induced typical facial expressions in a large number of examined subjects. We observed that these grimaces had a constant appearance and were found to be characteristic of each of the three taste qualities.

The consistent reaction which we first observed was that application of bitter stimuli to the tongue elicited a facial expression characterized by depression of the angles of the mouth, contraction of the platysma muscles, and protrusion of the tongue: an expression typical for disgust or aversion. Sour stimuli very often elicited a brief flushing of the face, contraction of the orbicularis oris muscle with protrusion or pursing of the lips, increased swallowing (indicating salivation) and often a brief transient wrinkling of the nose. Finally, we observed that application of sweet stimuli led to the appearance of a marked relaxation of the facial muscles, like a slight smile, resembling satisfaction or an expression of acceptance.

The examined subjects were of either sex, of different ages, of diverse ethnic, cultural and educational background, appropriate to the variegated population in Israel. We observed that the facial expressions appeared with a very short latency after application of the sapid stimulants. As a rule, they preceded the verbal answer of the taste recognition.

Some examinees felt embarrassed by the "grimaces", which appeared to be irresistible, or "automatic".

The consistency of the facial reactions, their short latency, their automatic appearance led us to assume that these facial expressions induced by sapid stimulants must be considered a cliche of behavior. That this is a reflex probably innate in type, rather than acquired by conditioning or learning. We decided, therefore, to test this assumption by stimulation of newborns, thus to learn about the innate character of the response, and to extend the study to animals in order to demonstrate whether a laboratory mammalian model could serve as an appropriate tool for further understanding of the neural control of the observed reflex-like response.

SUBJECTS, MATERIALS AND METHODS

The study was carried out on 179 neonates of either sex. The tested babies (with exception of two) were born in the Department of Gynecology and Obstetrics of the Hadassah University Hospital, Jerusalem. This population of examined newborns is divided into three groups:

A. 100 termborn normal neonates, born by normal delivery and found to be healthy by the routine check-up of the Unit of Newborn Child of the Department of Pediatrics. The test was carried out in this group between the 3rd and the 7th day of life.

B. 75 termborn normal neonates, born by normal delivery and found to be healthy by routine check-up. The test was carried out on this group between birth and the first breast or bottle feeding; this in order to exclude any previous experience with food intake and related taste sensations.

C. 4 newborns with developmental malformations of the CNS*. Two of these were cases of anencephaly, with hypoplasia of the skull. These neonates, both females, were tested between the 2nd and 6th day of life. Both of these neonates died in the hospital before they reached their 11th day. Autopsy was performed in both. The functional and developmental state of the nervous system of each was systematically studied. The two other malformed newborns were cases of hydrencephaly. The facial reactions of one of them was tested and filmed at the age of 19 days. The same newborn was examined also by radiography. This baby died away from the hospital and no autopsy was performed. The second hydrencephalic newborn was transfered from another hospital at the age of about 8 hours. This newborn was tested by us in the emergency ward of the hospital, without opportunity for further study.

Stimulants

1. Boiled deionized water was used as "tasteless" control stimulant.
2. 25% SUCROSE dissolved in boiled deionized water was used as "sweet" stimulant.
3. 2.5% CITRIC ACID dissol-

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*My special thanks to Dr. G. Szabo of the Department of Pediatrics; Hadassah University Hospital, Jerusalem, for turning my attention to the two cases of anencephaly described in this study.

ved in boiled deionized water was used as "sour" stimulant.

4. 0.25% QUININE SULPHATE dissolved in boiled deionized water was used as "bitter" stimulant.

In each stimulation, 0.5 ml volume of the stimulant was presented at room temperature by a disposable plastic pipette to the central area of the dorsal tongue surface. If the infants were in a sleepy state, we woke them up by noise or slight touching of the facial skin. Tests were not carried out during crying. The facial expressions were observed, recorded in standard protocol, and simultaneously recorded by videotape or motion picture. In some cases, only still photographs were taken. The stimuli were assigned by code, with random sequence of stimulus presentation.

The videotape recordings were played back and evaluated by a panel composed of a pediatrician, a child psychiatrist, a nurse, and a pedodontist. The experimentor did not take part in the evaluation panel. The code of the stimuli was not known by the panel members. We did not systematically calculate the correlation coefficients between the judgments of the experimentor and of the panel in all cases. In most cases the correlation was about 0.75–0.85. The correlation between the judgments of the members of the panel was so consistent that these calculations were omitted.

RESULTS

The observations made by the experimentor at the time of stimulation and the judgements of the panel evaluating the video or cinema recodings may be summarized:

1. The preponderant majority of the tested termborn normal babies of either age responded to sapid stimuli by clear facial expressions. The expressions differed from the licking, sucking and swallowing movements often induced by rinsing with tasteless water.
2. Each of the three taste modalities employed induced a facial reaction which was typical of the particular taste. The facial reactions as observed in the normal termborn babies of group B are shown in Figures 17–1—17–5.

a. Application of sweet stimuli led to the facial expression of retraction of the mouth angles; by this movement the lips became thinner, the relaxation of the other facial muscles associated with this retraction resulted in a smile-like expression. This facial expression was, in general, followed by very eager sucking with protrusion of the tongue tip to the upper lip; this movement was accompanied by a typical noise of sucking. After the newborn swallowed the sweet substance, a marked restlessness very often appeared. This was interpreted as "disappointment", caused by a "too brief" transition of the sweet perception. Repetition of the stimulation by the sweet substance led again to relaxation and reappearance of the expression of satisfaction, resembling smile, as well as sucking.

b. Application of sour stimuli induced a different type of facial expression, characterized by pursing and marked protrusion of the lips. The tongue became visible by stretching out; it was then rolled.

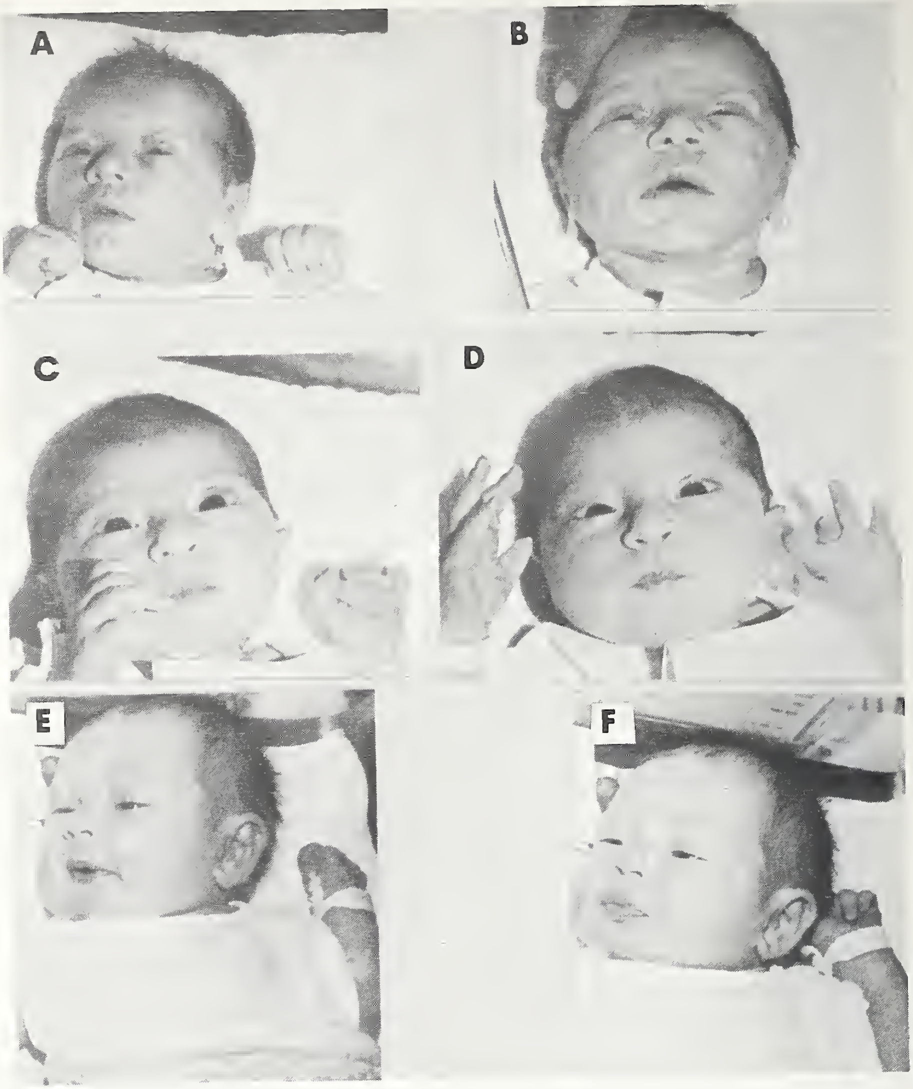


Figure 17–1. Facial Expressions of Normal Infant Without Tastant Stimulation.

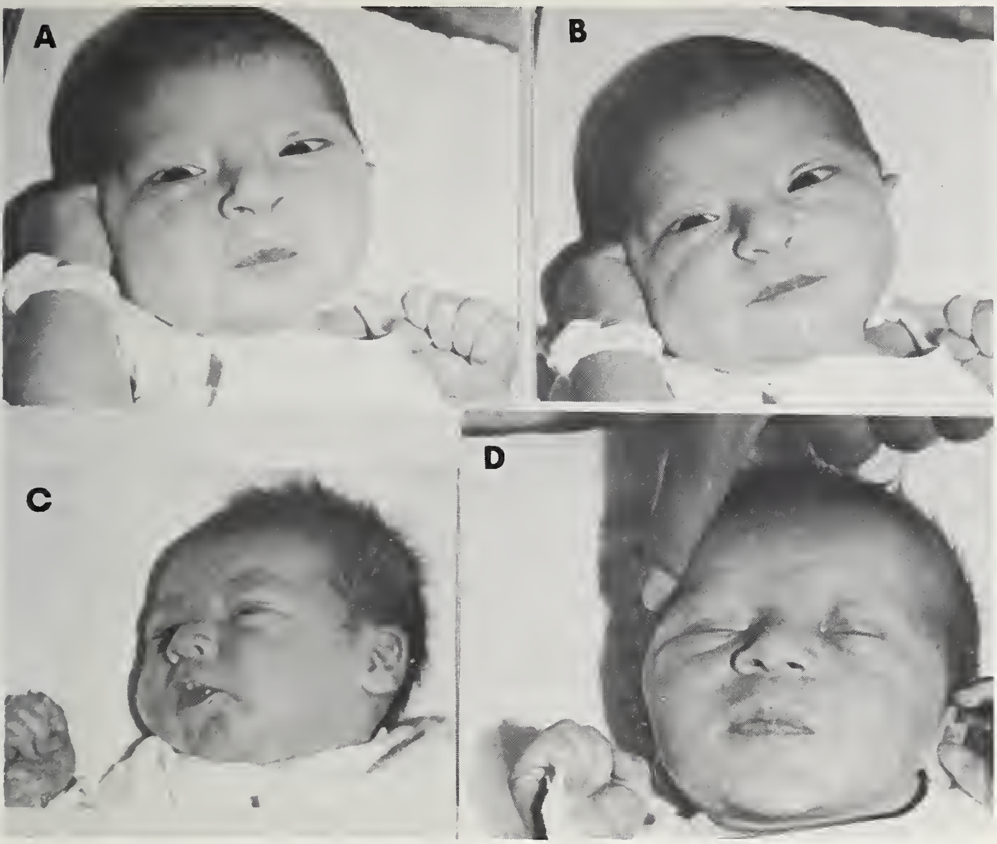


Figure 17–2. Gustofacial Response of Normal Infants to the Administration of Distilled Water.
No typical facial expressions are elicited by "tasteless" (control) stimulation.

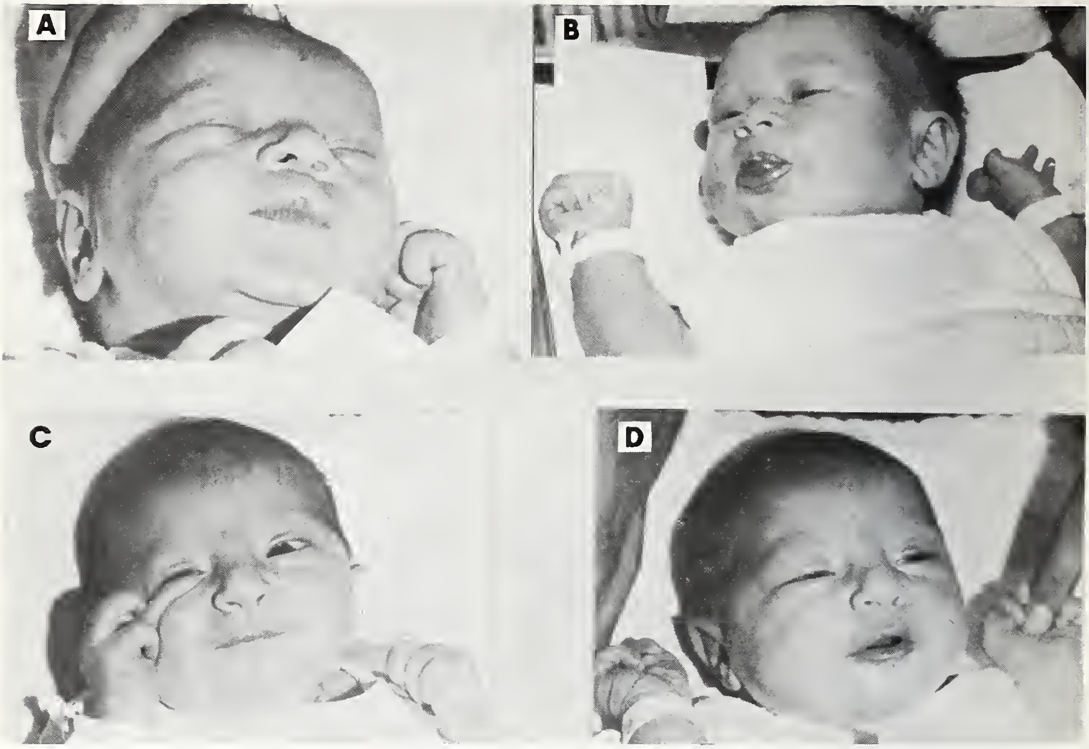
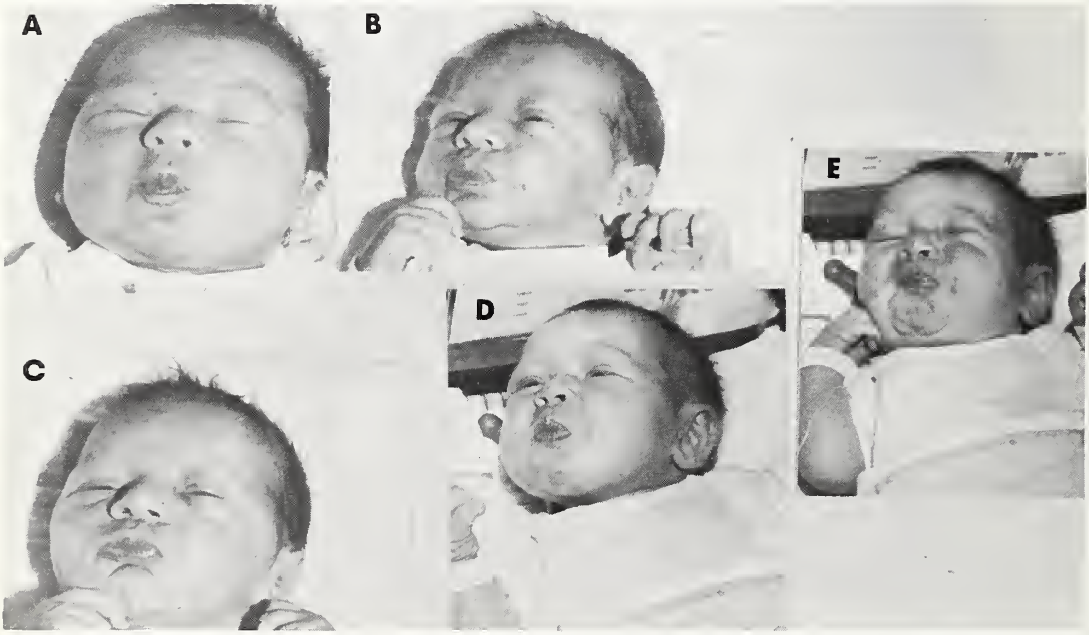


Figure 17–3. Gustofacial Response of Normal Infants Following Administration of Sweet (25% Sucrose solution) Tastant.
Expressions of relaxation (A) "satisfaction" (D) and eager sucking (B, C) are typical components of the "sweet response". This feature of expression resembles "smile".



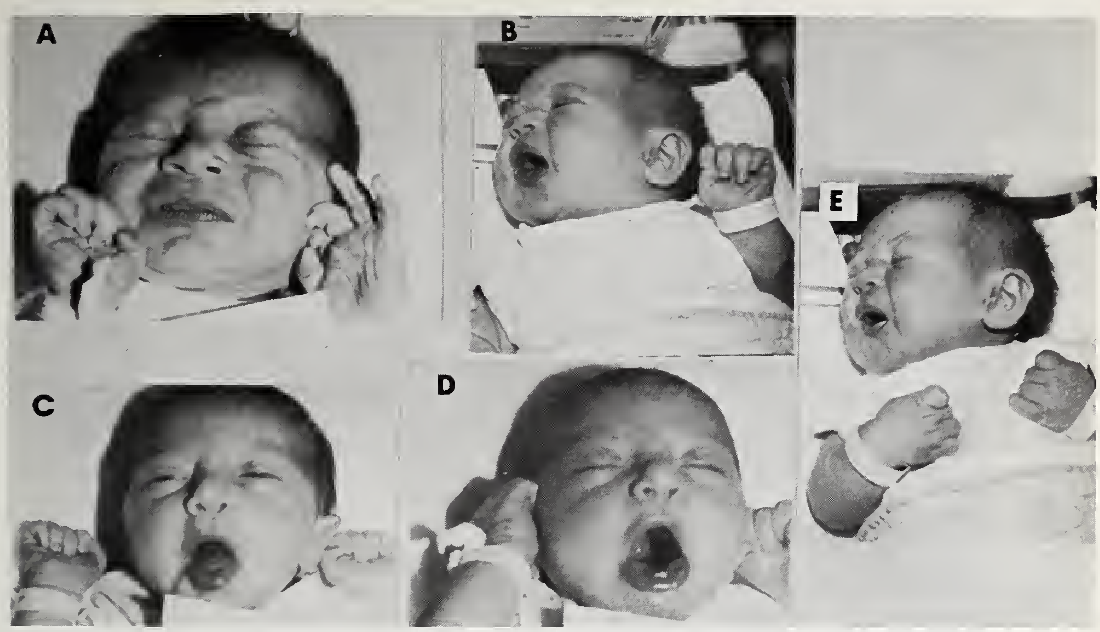


Figure 17–5. Gustofacial Response of Normal Infants Following Administration of Bitter (0.25% Quinine Sulphate) Tastant.
The "bitter response" is characterized by wide opening of the mouth, "arch-formed upper lip" (B-E) and by expression of "dislike", or "aversion", resembling expression of "anger".

≪ Figure 17–4. Gustofacial Response of Normal Infants Following Administration of Sour (2.5% Citric Acid) Tastant.
Protrusion of the lip and contraction of orbicularis oris muscle are the most typical components of the "sour response" (A-E); frequent, repetitive blinking (D) and wrinkling of the nose (E) are other components of this "sour response".

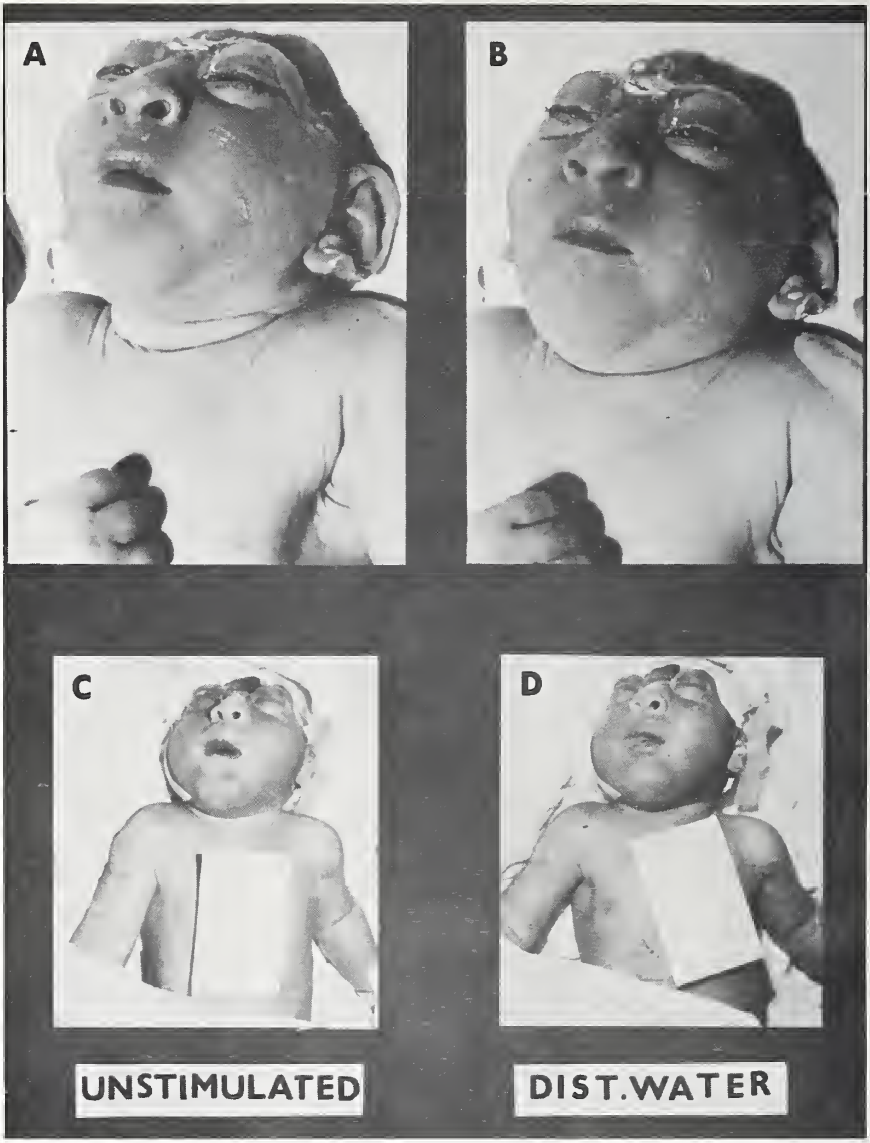


Figure 17–6. Gustofacial Response of Anencephalic Infant Following Administration of Distilled Water.

Resting face (A and C) and face after stimulation with distilled water (B, D) as control.

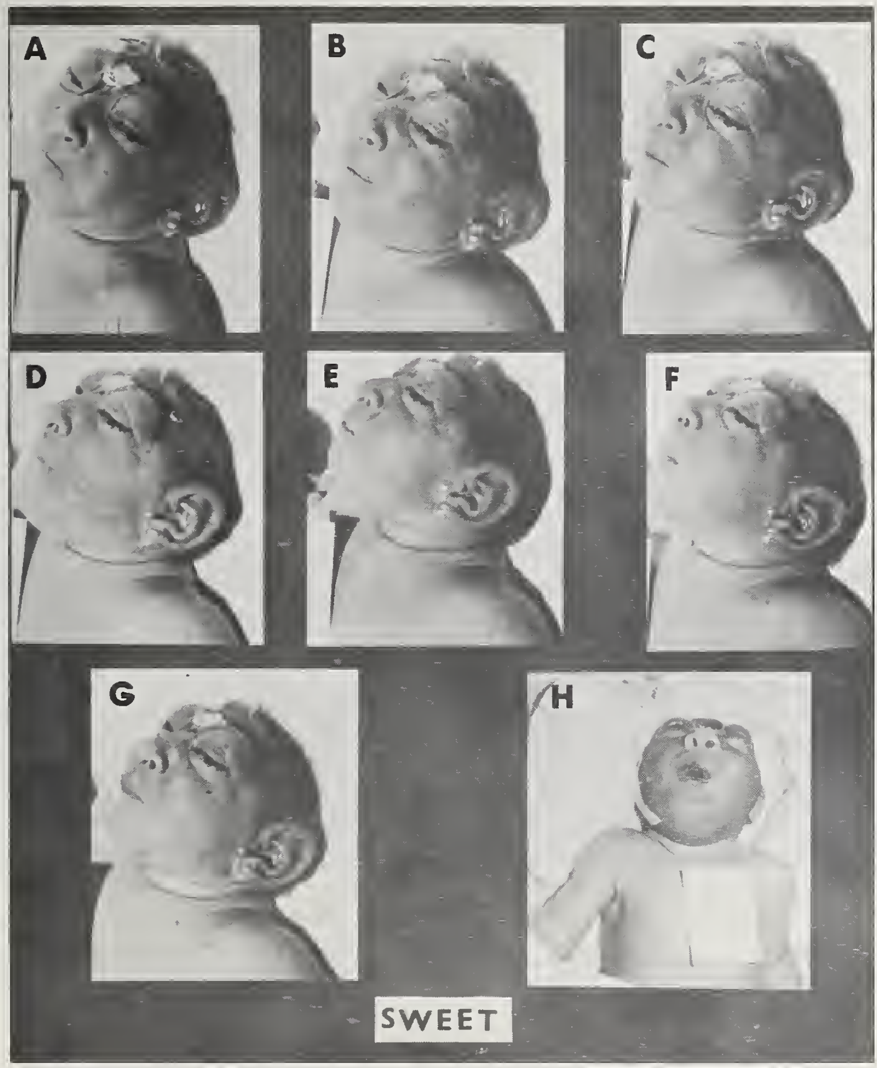


Figure 17–7. Gustofacial Response of Anencephalic Infant Following Administration of Sweet (2.5% Sucrose) Tastant.
The typical festures of "sweet reaction" are shown in a sequential series of pictures (A-G) taken from the first case. Note the "relaxation" resembling "smile" in B, D, E and F. The sweet-induced sucking is clearly visible in H.

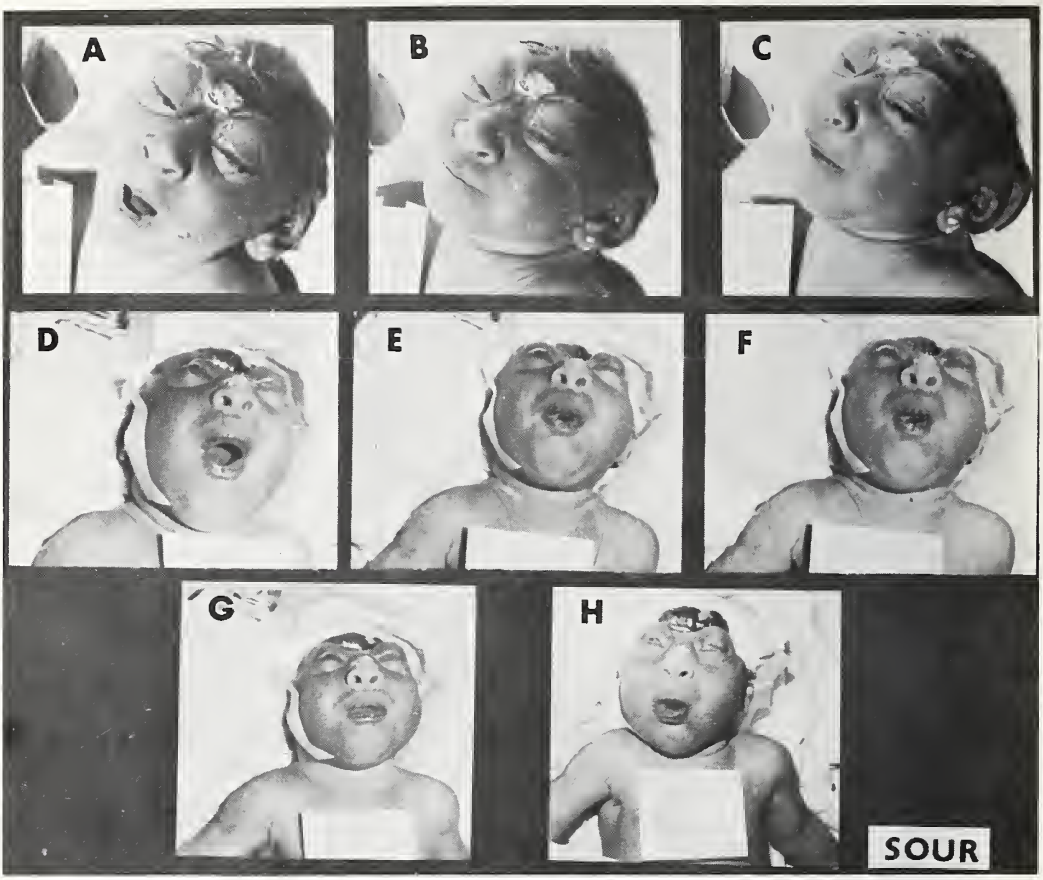


Figure 17–8. Gustofacial Response of Anencephalic Infant Following Administration of Sour (2.5% Citric Acid) Tastant.
In sequential series of pictures taken from both anencephalic cases (A-C and D-H, respectively) the appearance of lip-pursing due to "sour" stimulation is shown.

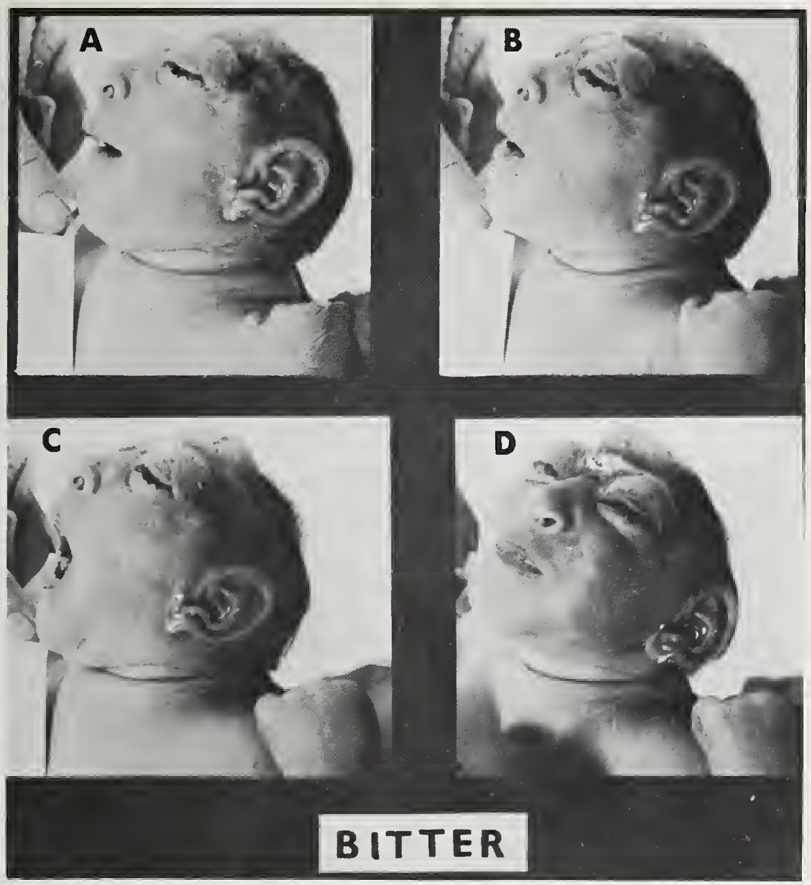


Figure 17–9. Gustofacial Response of Anencephalic Infant Following Administration of Bitter (0.25% Quinine Sulphate) Tastant.
The depressed lower jaw and expression of "dislike" are demonstrated in photos A-C, salivation and "spitting" are demonstrated in D.

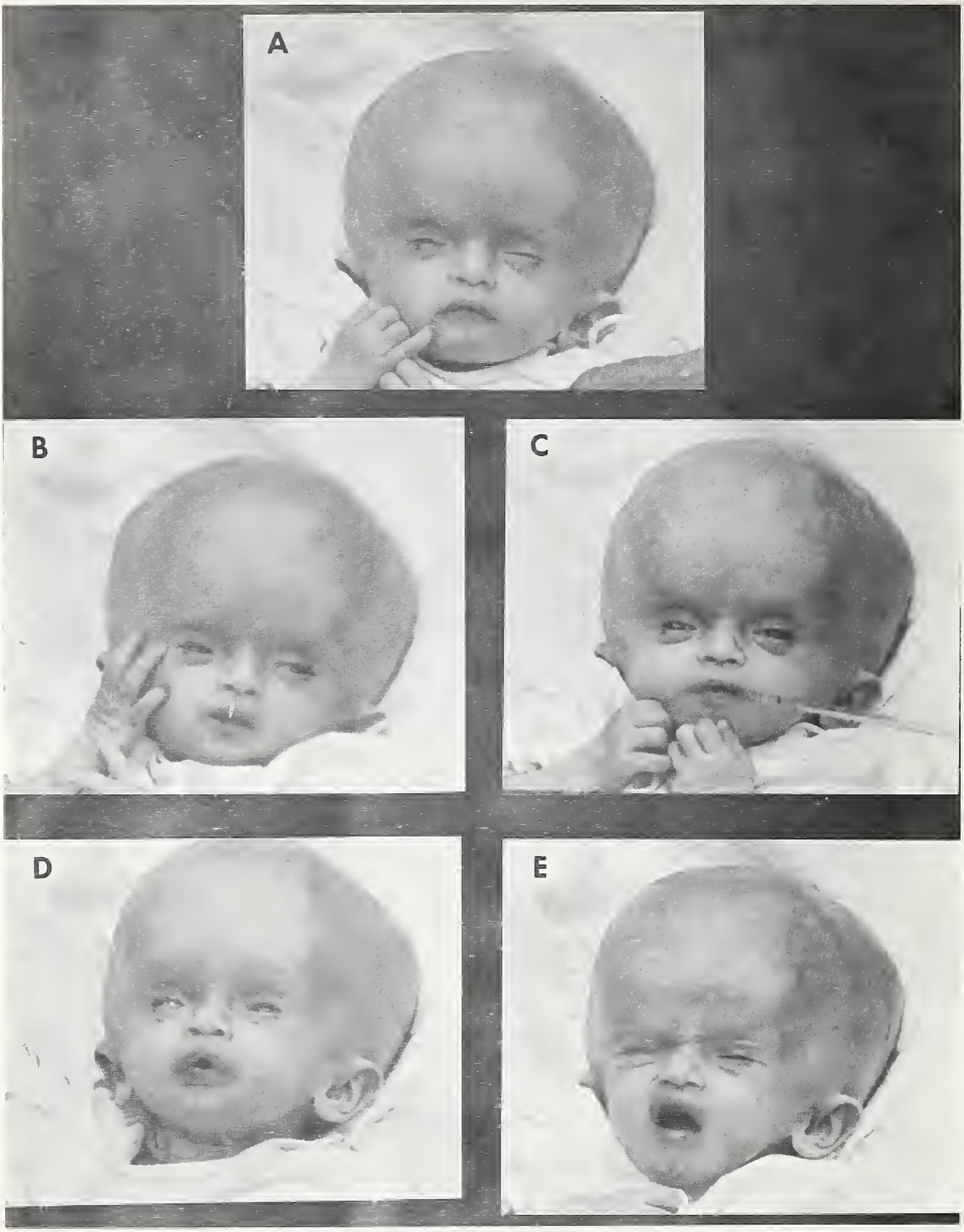


Figure 17–10. Gustofacial Responses of a Hydrencephalic Infant
A - Resting face, without stimulation
B - After presentation of distilled water (tasteless)
C - Response to "sweet" tastant (relaxation)
D - Response to "sour" tastant (lip pursing)
E - Response to "bitter" tastant (aversive reaction)

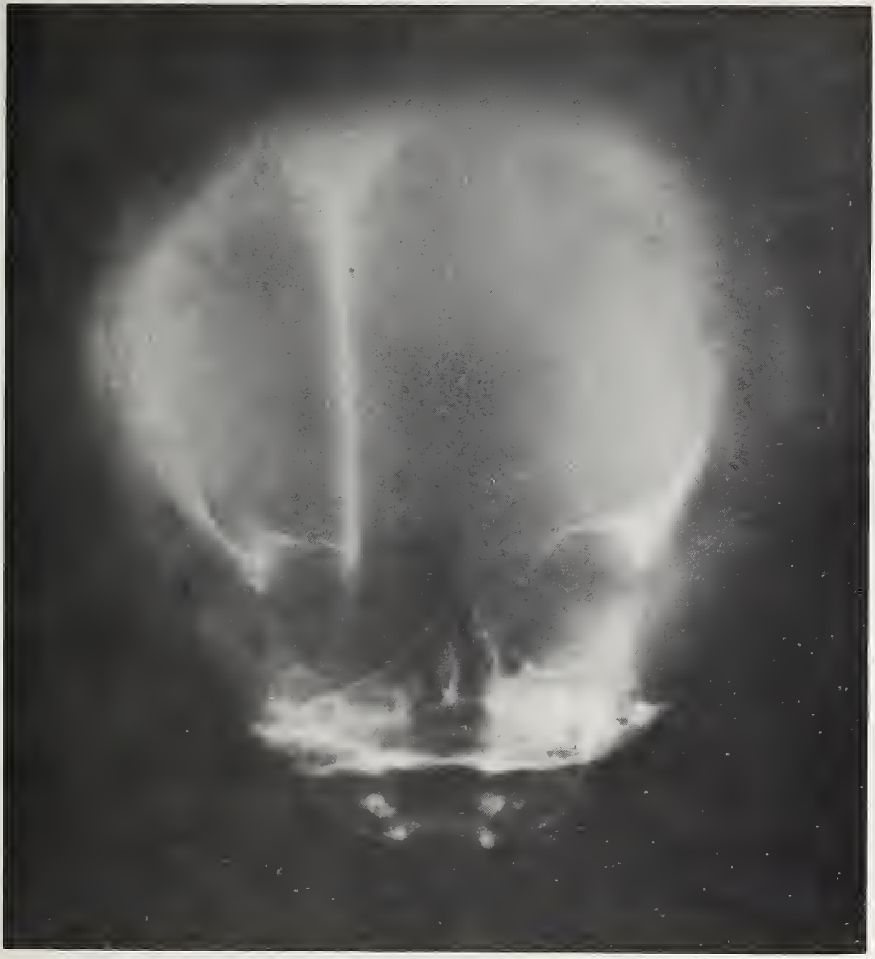


Figure 17–11. Anteroposterior Radiograph of a Hydrencephalic Neonate.
Note enlarged, globular calvarium, and the displacement of the radiodense falx to left of subject's midline.

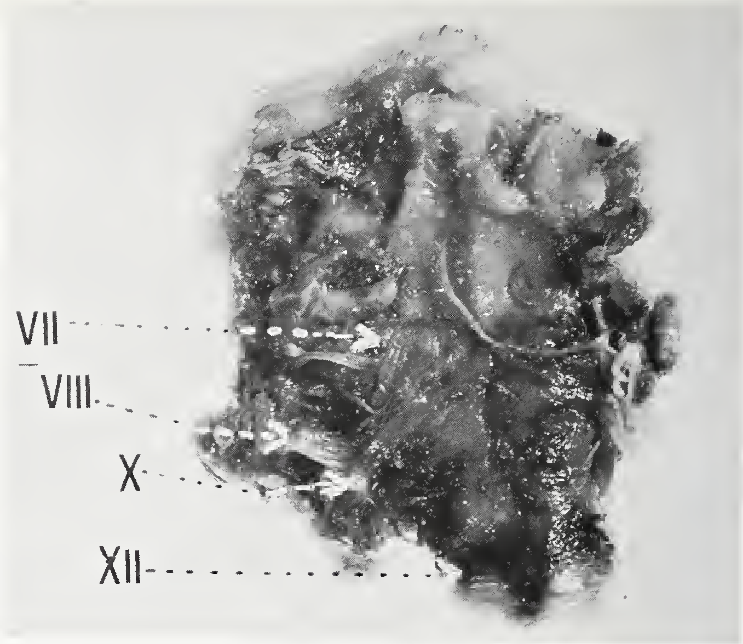


Figure 17–12. Ventral view of the brain stem of one of the tested anencephalic neonates. The photo shows the brain stem after removal of the cyst and of the parts of the cerebellum which were found to be present in this case.

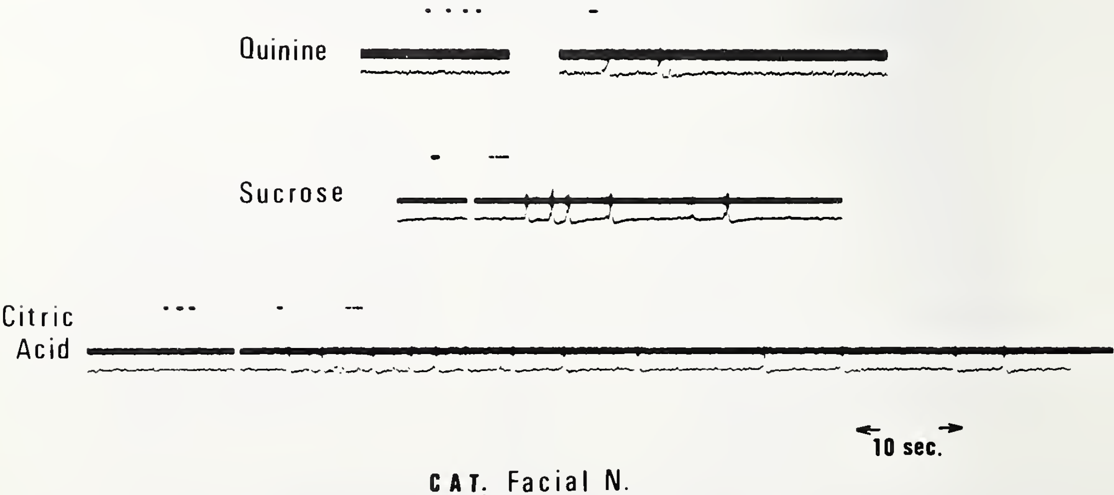


Figure 17–13A. Recordings from the Facial Nerve of an Anesthetized and Immobilized Cat.

Upper trace: recorded neural activity; lower trace: integrated activity. Application of quinine to the tongue leads to a slightly increased activity. Sucrose induced more activity. Citric acid induced the greatest neural response to taste stimulation.

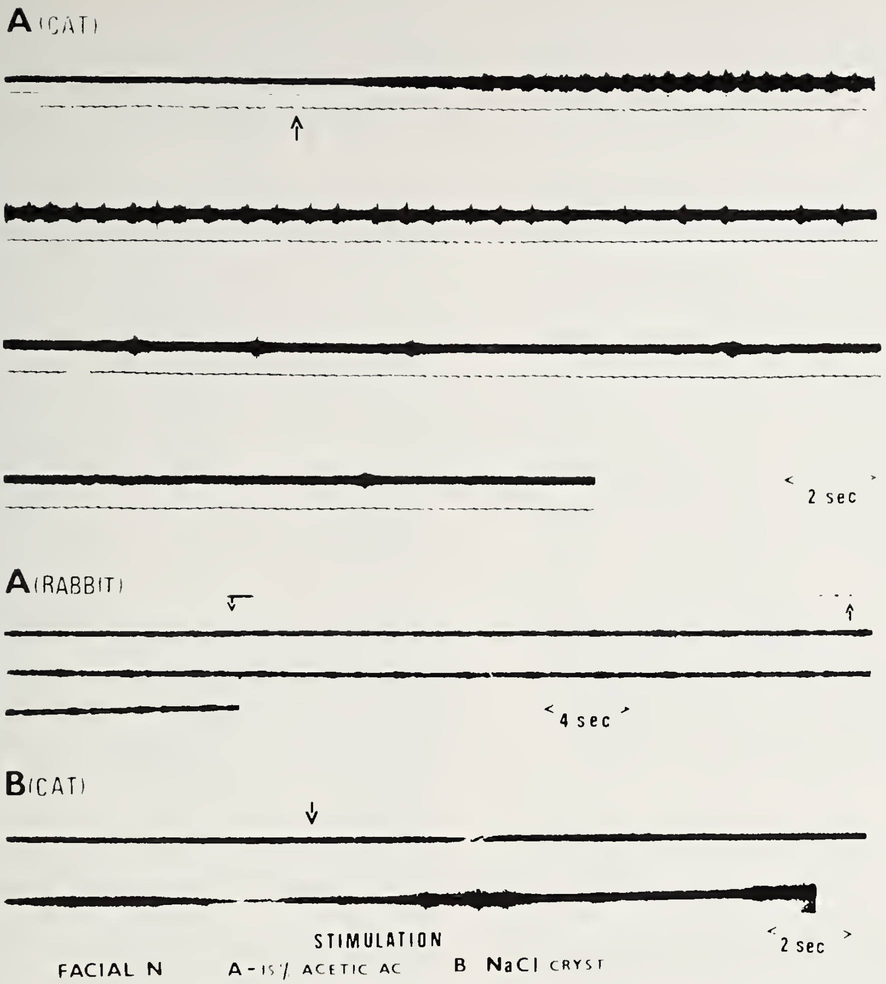


Figure 17–13B. Recordings from the Facial Nerve in Cat (A) and in Rabbit (B) after Taste Stimulation of the Tongue.

The rinsing of the cat's tongue leads to the appearance of burst activity. The lower tracing is ECG.

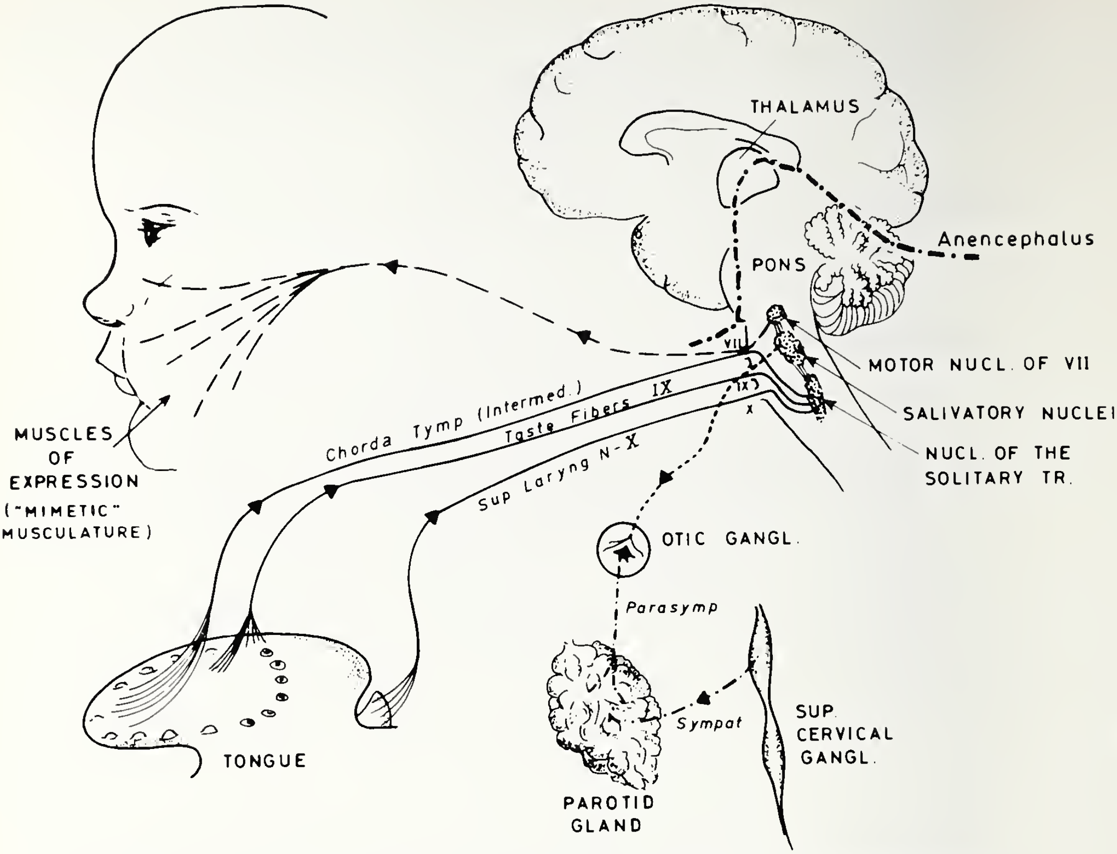


Figure 17–14. Proposed Neural Pathology of the Gustofacial Reflex, as Demonstrated in the Human Neonate.
The level of ablation of the CNS in the anencephalic infants of this study is indicated schematically by the interrupted line.

The pursing could be a steady, long-lasting holding of the position, or could be repeated very frequently, with a twitching element of the movement.

The lip pursing which is the most characteristic expression component of the reaction to sour could be accompanied by a long-lasting or repeated wrinkling of the nose, with or without simultaneous blinking. Two vegetative responses were observed as typical sequellae of sour stimulation: increased salivation, (evidenced by drooling) and briefly transient flushing of the facial skin.

c. The application of bitter stimuli induced a third type of response. This was characterized by marked depression of the mouth angles, with simultaneous elevation of the central part of the upper lip, and stretching of the lower lip. The platysma muscle contracted and thus the open mouth had an arch–like appearance. The tongue, which became visible in this position, was always flat and slightly stretched out. Movements of spitting, accompanied with increased salivation, appeared later, giving the face an expression of dislike, aversion and anger. In a large number of examined newborns, slight vomiting movements were induced, particularly in the babies of group "B."

3. When the stimulus was repeated, either after rinsing with tasteless water or after another sapid stimulation, facial expression typical for the particular taste could be evoked again. Essentially no "exhaustion" of the evoked facial response could be observed.
4. The observed typical "grimaces" induced by the modalities of sweet, sour and bitter appeared regardless of the sequence of presentation.
5. Some preliminary observations were carried out on premature neonates in which only the sour stimulation was applied. These showed that about 20 infants born during the 7th month of pregnancy produced the typical "sour response": pursing of lips and nose wrinkling. (This observations was made by a colleague in the Newborn Unit in the Central Negev Hospital of the Sick-Fund in Beer Sheva, Israel)

From these results of the stimulations of the Group "B" neonate it is inferred that these patterns of facial expression are innate.

OBSERVATIONS OF INFANTS WITH DEVELOPMENTAL MALFORMATIONS OF THE CNS

Gustofacial responses were elicited and photographed in the 4 infants with severe CNS malformations. The severity of the CNS malformation of one of the hydrencephalic infants is indicated in the anteroposterior radiograph of the head in Figure 17–11.* This demonstrates a laterally displaced falx, indicative of the large cyst which occupied nearly the whole cranial cavity.

The brain of one of the anencephalic neonates is illustrated in Figure

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*Footnote: This radiograph is presented here by the courtesy of Dr. A. Sahar, of the Department of Neurosurgery in our Medical Center.

17–12.** The myelencephalon was found more or less intact, and some parts of the cerebellum and the diencephalon were present. No other higher structures could be recognized. This photograph shows the cranial nerves VII, VIII, X, and XII, indicated with arrows.

The hydrencephalic newborns had normal facies despite the disproportionately large and globular calvarium, so that the expressive movements in the facial region were easy to recognize. The unusual facies (the "frog-like face") of the anencephalic neonates did not make it difficult to recognize the typical features of the taste-induced expressive movements. The reactions of these four infants to sapid stimuli are illustrated in Figures 17–6—17–10. Tasteless and sapid stimulants elicited facial expressions similar to those in the normal babies. To quantify observations of the experimentor and the judgements of the evaluating panel, we simply counted the frequency of the appearance of the most typical features of the facial expressions evoked by each of the three applied taste stimuli. The results are presented in Table 1. Statistical analysis was not indicated.

A MODEL OF THE GUSTOFACIAL RESPONSE IN LABORATORY MAMMALS.

Experiments have been carried out on 15 cats and on 10 rabbits of either sex. The animals were anesthetized by a special combination narcosis developed in our laboratory for neurophysiological experiments.[29] To avoid movements in the facial region and invasion of EMG activity into the recording, the animals were slightly curarized and artificially respired. Under anaesthesia, the peripheral portion of the facial nerve in the region of the masseter muscle and parotid gland was exposed and placed on recording electrodes. The gross neural electrical activity was amplified and displayed on an oscilloscope, monitored by a loudspeaker and recorded by a Schwarzer PHYSIOSCRIPT polygraph. The preamplified signal was also integrated and recorded on the polygraph. The neural activity was recorded under unstimulated conditions for control purposes, as well as after rinsing of the tongue by the same sapid stimulants used in the human experiment. Typical records are shown in Figure 13. In 12 of the 15 cats, the application of sapid stimuli to the animal's tongue led to a marked increase of the efferent electrical activity carried by the facial nerve. Similar results were obtained in 7 experiments of the 10 rabbits. The increased activity observed was of two types: In some it was continuous. In others, especially after the application of sour stimuli, the centrifugal potentials appeared in "trains" with intermittent silent periods. The induced activity appeared with a very short latency and diminished progressively in 90-120 seconds. In 3 experiments, NaCl crystals placed on the tongue elicited neural activity at longer latency. This longer latency was seemingly caused by the time necessary for solution of the crystals.

The facial motor response to taste stimulation in these laboratory animals were evaluated only by neural recording. The movements of the face or of

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**Footnote: This photograph is included by the kind permission of Dr. A. Ornoy of the Department of Anatomy in our Medical School, who carried out the pathological and developmental studies on both of the anencephalic neonates.

FREQUENCY OF APPEARANCE OF THE MOST CHARACTERISTIC FEATURES COMPOSING FACIAL RESPONSES TO GUSTATORY STIMULI

	Tested Neonates		
	TERMBORN NORMAL		ANENCEPHALIC CASES
	age 3 - 7 days	age below 20 hrs	age 1 - 19 days
	n = 100	n = 75	n = 4
SWEET			
RETRACTION OF MOUTH - ANGLES	87	61	4
CLEAR EXPRESSION OF SATISFACTION RESEMBLING "SMILE"	73	58	3
EAGER SUCKING WITH LICKING OF THE UPPER LIP	97	74	4
SOUR			
PURSING OF LIPS	98	75	4
WRINKLED NOSE	73	58	2
QUICK REPEATED BLINKING	70	67	2
INCREASED SALIVATION	65	61	4
FLUSHING	64	57	1
BITTER			
"ARCH-LIKE" LIPS WITH DEPRESSION OF MOUTH ANGLES	96	73	4
PROTRUSION OF FLAT TONGUE	81	59	3
SALIVATION AND SPITTING	87	57	4
GENERAL EXPRESSION OF "ANGER" AND "OISLIKE"	86	59	3
VOMITING	52	34	3

particular facial muscles were not analyzed.

Within its limitations, this model indicates the existence of gustofacial reaction in lower mammals which is homologous to that which has been observed in the newborn and in the adult.

In general summary of these results: Suprathreshold stimulation of the human tongue with sweet, sour, and bitter stimuli elicits facial expressive movements which differ markedly from the facial expression induced by tasteless stimuli and are characteristic for each of the three particular taste qualities applied. The reactions were found to be of fixed, rigid cliches of expressive movements. That the reaction is elicitable in the earliest phase of extrauterine life indicates the innate character of the response. The elicitation of taste-specific responses in these malformed neonates indicates that this catalog of responses resides in the brainstem. The findings in the animal experiments support the concept that the gustofacial response is a general phenomenon within the mammalia.

Concluding from these results, we propose the following pathway of this reflex (a) peripheral sensors of taste on the tongue and pharynx (b) afferents carried by the chorda tympani (n. intermedius) and by the sensory fibers of cranial nerves IX and X, (c) medullary. evaluation of the incoming taste-signals in the nucleus of the solitary tract (d) fiber connections to the motor nucleus of the VII nerve (e) activation of the expressive musculature of the face. This proposed pathway does not involve any higher (cortical) participation, and therefore the existence of the reflex in the anencephalic neonates with remainders of brain-stem can be easily understood. The proposed pathway is shown in Figure 17–14.

The neural pathway of taste–induced salivation is very similar. The incoming signals are transferred by fiber connections from the nucleus of the solitary tract to the salivatory muclei (lower and upper). It is not surprising, therefore, that simultaneously with the gustofacial reflex drooling is also present especially in the neonate.

From the communication point of view the gustofacial reflex belongs to the nonverbal catalogue serving seemingly primarily the child-mother interaction, in terms of "acceptable" or "to be rejected", respectively, as related to food intake.

DISCUSSION

The lingual papillae were described by Malphighi in 1664.[18] Schwalbe[27] and Loven[17] in 1867 recognized the buds as the receptors of taste. Subsequently, there have been several studies of the embryological development of the taste buds. Recently we had the opportunity to examine a large number of human fetal tongue samples both by conventional histological methods and under the scanning electron microscope (SEM).[32] These observations have shown that the adult form of the human taste bud is clearly visible in histological preparations of the human embryo in the 5th gestational month. At this age, SEM demonstrated open pores of the buds. This morphological observation fully agrees with the findings of Bradley and Stern.[3]

From the morphological evidence of Bradley and Mistretta[4] in animals, the obstetrical observations of De Snoo,[6] and the observations by Windle[36] in the human embryo, it may be inferred that the gustatory apparatus is well developed and in functional state long before gestational term. The demonstration here reported of competent discrimination of sour taste in infants born prematurely in the 7th gestational month further validates and specifies this inference.

A decade after Darwin's "Expression of Emotions . . ." was published, a German physician, Genzmer[11] systematically studied processes of sensory perception in newborn infants. Genzmer[11] had been alerted to the importance of investigating these functions by an earlier study of Kussmaul.[14] These investigators recognized that observations of the newborn's ability to react to sensory stimuli was an "appropriate approach to an insight into the psychic life of the newborn".

Genzmer[11] stimulated 25 newborns with sweet, sour or bitter solutions, using sucrose, acetic acid and quinine sulphate as stimulants. He described, in accordance with the observations of Kussmaul, typical grimaces induced by strong sweet, strong sour and strong bitter stimulation. The facial expression induced by sugar was described as sucking with an expression resembling that of enjoyment and pleasure in the adult. The application of sour elicited a typical facial expression described as the "sour face" of the adult. The application of bitter induced typical movements of the facial musculature, an expression resembling the "bitter face" of the adult, with wide opening of the mouth. The bitter stimulant solution caused profuse salivation and was spat out. Our observations regarding the taste-induced facial expression are in agreement with those of Genzmer.

It seems to us that the most striking point in the findings of the present study is that the gustofacial reflex demonstrates the ability of the ponto-medullary region in the human brain to discriminate between sensory signals, announcing that some events are acceptable or welcome for the organism, and others must be rejected, as harmful or noxious. People are often inclined to believe that discrimination between "good" and "bad" is mainly a cognitive function based on life-experience conditioning, learning and an emotional attitude. However, Hess,[13] in extensive stereotactic electrical stimulations in the midbrain distinguished loci which controlled beneficient processes, such as food and fluid intake, sleep, relaxation, etc. In other loci, he elicited protective behavioral patterns related to elimination of harmful events in normal life situations. One of these patterns which Hess elicited in the anterior thalamus was "expulsion of a disturbing object from the oral cavity". He also filmed the full sequence of these movements, starting with retraction of the mouth angles and wide opening of the mouth, followed by abrupt forward propulsion of the tongue.*

With all precautions about homologic comparisions, it is still appropriate to note the similarities between these facial movements elicited in the cat thalamus and the facial expression of normal and anencephalic neonates to presentation of bitter stimuli.

Discrimination between good and bad is one of the basic tasks in the achievement of optimal living conditions. The reflex-like search for optimal conditions can, therefore, be considered a special situation of homeostasis. It seems clear that reflex processes operating at different levels of the CNS must be responsible for this "optimostasis". It is suggested that noncognitive discriminations between events indicating "good" or "bad" for the organism are represented at different levels in the highly developed vertebrate CNS. Similar discriminative abilities are present in much lower phylgenetic ranges. It is inferred that the phenomena termed "taxis" or "tropism" and found in very primitive living forms are also to some extent discriminative processes.

In terminology used by the human ethologists such as Peiper,[21] Eibl-Eibesfeldt,[7] Lorenz[16] and others, the gustofacial response is an innate behavior, as inherited motor coordination. When this response is classified according to its biological function, it belongs to the group of innate motor coordinations related to search for food and food intake. Innate motor coordinations of this type have been described in animals and in man by Prechtl[26] and by Prechtl and Schleidt.[25] Pilleri[22,23] characterized the innate coordination cliches according to the following criteria: (a) they are rigidly fixed in their appearance, (b) they are resistant to exhaustion by repeated elicitation, (c) they are homologically distributed. If an innate coordination is found in man, a similar coordination will also be found in lower mammals, at least in primates. The gustofacial response seems to fulfill each of these criteria.

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* Special thanks is given to Prof. M. Monnier, head of the Department of Physiology, Faculty of Medicine, University of Basel for his kindness in projecting for me the original motion picture records of this reaction, described by Hess. Prof. Monnier is a former co-worker of W.R. Hess.

A large number of neurological phenomena have been examined in anencephalic neonates.[8,10,19,20,34] Gamper,[10] Monnier and Willi[19,20] and others pointed out that anencephalic neonates make "grimaces" following strong taste stimuli, but reported no clear generalizing description of the taste-induced facial expressions. These malformed newborns are unique models, not producible in any laboratory experiment. Specifically, the anencephalus is superior to the experimental situation produced by decortication of a laboratory animal, because such an experiment can never annual the effects of the previous role of the higher brain structures. In the anencephalus, such higher brain structures have never been functional, because they have never developed. The two anencephalic and two hydrencephalic neonates of this study demonstrate that this response can be effected by the brain stem which has not interacted with higher cephalic structures.

Mimicking is one of the richest and most differentiated repertoire of nonverbal expressive movements and communication systems with which man is equipped. The term "mimic" refers to the general belief and assumption that this form of communication signals is learned by processes of imitation. It is well known today, that this assumption is not valid. We must change our concept, and consider the basic features of the human facial expressions as innate stereotypes. For example, the smile was believed to be imitated by the newborn. But Eibl-Eibesfeldt[7] observed that smile appears as a spontaneous motor reaction without external stimulus. Freedman[9] demonstrated smile in the blind neonate, who is obviously unable to imitate the smile of the adult face.

The very complicated and complex repertoir of adult facial expressions is a product of life experience including conditioning, learning, etc., which activates, facilitates or inhibits the existing, innate or even inherited basic features of the catalog of human facial expression. These innate motor coordinations can be activated as reflexes by appropriate sensory stimuli. In the gustofacial response, a well defined sensory stimulus triggers an expressive movement feature. This response may be a useful model for the definition and study of other sensory induced facial reactions.

The modulation and, mainly, the *inhibition* of these reflexes and their transformation into indicators of fluctuations of moods and feelings is seemingly a maturation achievement of the frontal lobe. The bibliography of this inhibitory or "damping" ability of the frontal lobe in the human brain is extensive. It is particularly pertinent to point to Bracha's description[1,2,35] of several neurological signs related to the perioral area, which are accepted pathognomonic signs of frontal lobe disturbance.

I would propose that the gustofacial response is also inhibited during postnatal development. This may explain the less characteristic and less consistent responses found in the adult compared with those in the neonate. Conversely, the distinct and consistent gustofacial responses to gustatory stimuli in the *anencephalic* neonate may be due to the absence of inhibitory elements in the malformed brain of these neonates.

It follows from these considerations that the gustofacial response should appear distinctly and consistently in pathological cases in which other signs of frontal disinhibition is diagnosed as sequel of frontal lobotomy or other lesions of this area, or in cases of incurable psychosis. Reappearance of the gustofacial response in its infantile form may serve as an indicator of regression, comparable to reappearance of the sucking reflex induced by tactile stimulation of the upper lip, as in severe cases of schizophrenia. The systematic examination of this response in a population of psychotic patients, especially in cases with severe regression signs, offers interesting field of study, which we plan to investigate in the future.

In reciprocal perspective, consecutive examination of this response and other stimulus–elicited facial expressions may offer a route of study of normal and of abnormal psychological development of the child.

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Chapter 18

EFFECT OF TASTE ON INGESTION BY HUMAN NEWBORNS

Owen Maller and J. A. Desor

INTRODUCTION

The flavors of food are important determinants of selection and ingestion by man and animals. More information about the contributions of taste and odor to alimentation is available for subhumans than humans.[12] The relative ease with which a diet can be flavored and the consumption by animals measured has facilitated their study. Our knowledge about the functioning of the senses of taste and olfaction in human feeding is anecdotal and fragmentary. Studies are reviewed that suggest the functions that taste may serve in the control of ingestion. Our studies of these functions in the human newborn are then described.

HISTORY AND BACKGROUND

Formal study of perception of tastes and odors by man began in the 19th century. Receptors involved in savoring were identified and classification schemes were evolved to describe and analyze these sensations.[4] Methods have been developed to quantitatively measure human sensitivity, discriminability, and intensity of chemical stimuli in air and fluid media.[10] Modifications of these methods have been used to evaluate the acceptability of flavors of foods and beverages.[3] Underlying these research efforts is the assumption that such indices of sensory perception may be useful in predicting selection and acceptance of nutriments. To date, there are only a few studies that deal with the problem of validating these indices (sensitivity, discriminability or preference) with direct measures of selection or ingestion of food.

A selective review of the nutritional literature suggests that the sensations of taste and olfaction serve several functions in the feeding process. Sensations of savoring are involved in 1) discrimination of foods, 2) selection of foods and 3) control of quantity of food consumed.

Flavor as a cue in the discrimination of foods

Harris et al,[16] in studying Vitamin B_1 deficiency in rats, observed that de-

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pleted animals selected a diet containing the vitamin, but normal animals did not. The thiamine-deficient animals could be educated to select the Vitamin B_1 diet from among a large number of diets on the basis of a distinctive flavor added to it. When the flavor associated with the repleted diet was shifted to another diet devoid of the vitamin, the animals tended to follow the flavor and the vitamin deficiency redeveloped.

Scott and Quint[39] studied the effect of flavor on selection of diets by young growing rats. They observed that "a fishy flavor" was avoided but that other additives were without any influence.

In an extensive study on the physiological factors affecting appetite for food and fluids, Adolph[1] examined the effects of flavor. He was interested in determining whether flavoring agents could induce an animal to injest nonnutritive materials such as clay and cellulose. Adding the sweetener saccharin led to a slight acceptance of these materials, but adding this flavor was not as effective as adding nutritive materials. In these experiments animals were able to discriminate between diets with and without nutritive materials. Adolph concluded:

> "Hence it seems difficult to fool a rat into accepting a non-nutrient by such means. Flavors that furnished nutrients in small amounts induced eating in close proportion to their nutrient contents taking chow as the standard of comparison. It is still possible that flavors may make a greater impression where choices among foods are allowed. It is also possible that more impelling flavors will be found." (p. 114)

Selection of nutrients

C. P. Richter demonstrated that flavors mediate selection and ingestion by the rat.[35,36] Adrenalectomized and pancreatectomized animals responded differentially to solutions of saline and glucose immediately following surgery. The change in hormonal levels produced dramatic changes in taste preferences for salt and sugar. In the case of the enhanced appetite for salt by the adrenalectomized rat, Richter[32] speculated about the mechanism of the altered appetite:

> "It has always seemed likely to us that appetite changes occur at the taste-bud membrane. Under conditions of salt deficiency, when all tissues of the body are in a state of salt deficiency, there is also less salt in the taste-bud side of the membrane. It follows that sodium ions will be transported across the membrane from the ingested solution to the cell fluids at a lower concentration than would be the case if the latter had a higher (normal) salt content. It is conceivable that when salt solution comes into contact with the taste-bud membrane, chemical changes are produced that make the salt much more recognizable (or attractive) than it would otherwise be if ample amounts of the salt are present in the blood." (p. 617)

He also conjectured that the differential appetites of an animal for nutrients served homeostatic functions.[32,35] These investigations have stimulated a number of researchers to examine taste and smell sensitivity of humans with pathological or normal variations in their internal chemistry, e.g., in the alteration of the levels of electrolytes or blood sugar. The results of these and other animal studies are contradictory and do not support any simple generalization about the relationship among the levels of these metabolities in the blood to taste sensitivity and appetite.[18,19,27,41]

In contrast to Richter's ideas about the function of taste in acceptance is the work of P. T. Young.[42] He stressed that the acceptance of foods is based on the immediate preferential response of the animal, which is elicited by the sensory qualities of nutrients, and that these preferences may not correlate well with homeostatic or energetic needs. To support this, Young offered rats a choice between a milk protein and table sugar. The animals showed a preference for sugar which persisted during a period of protein deficiency.[43] Young believed that the immediate preferential reaction of the organism determined whether a food is accepted.

Hedonic responses of humans to tastes and odors have been measured but their relationship to selection and ingestion of food has not been extensively elaborated.[11,29] Pilgrim found that he was able to predict what foods soldiers eat from their hedonic ratings of food items.[30]

Two studies of primates emphasize the importance of experiential and cognitive factors in the development of food preferences. Weiskrantz and Cowey[40] studied the acceptance of new food items that were introduced into the dietary regimen of rhesus monkeys. Both ingestive experience with the items as well as observing other animals consuming the new food were important for acceptance of the novel food by the monkeys. Duncker,[8] in a series of interesting experiments with pre-school children, demonstrated that observational learning and suggestion affected taste preferences. Children's preferences for certain foods were altered under the influence of either the observation of the selections of older children or the reported preferences of a major character in a story.

Flavor and the quantity of food consumed

Several studies with animals have shown that the quantity of food ingested is influenced by flavor,[12] but few studies have been done with humans. Nisbitt & Gurwitz[28] offered human infants special feedings of formula which contained 0.14 M sucrose or 0.20 M lactose. They reported that human neonates ingested larger quantities of an infant formula when sucrose was substituted for lactose, indicating that newborns perceive sugars, and prefer the formula flavored with sucrose.

The impact of flavor on the consumption of foods in infants is underscored also by the qualitative studies of Davis.[6] She studied the selection and consumption of thirty-four food items by young children offered these items in a cafeteria-like situation. From six years of observations, she concluded:

> ". . . patterns of selective appetite, then, were shown to develop on the basis of sensory experience, i.e., taste, smell, and doubtless the feeling of comfort and well-being that followed eating, which was evidenced much as in the breast-fed infant. In short, they were developed by sampling, which is essentially a trial and error method." (p. 10–11)

One of the few studies attempting to relate taste sensitivity to eating behavior is a study by Korslund & Eppright.[23] They obtained measures of sensitivity to sucrose, acetic acid, sodium chloride and quinine sulfate in children 49–61 months of age. These indices were then correlated with maternal reports of the amount of food eaten or rejected during a three-day period. They concluded that those children with the lowest indices of taste sensitivity tended to accept more foods and to have more enthusiasm for food than did those individuals who had the highest taste sensitivity.

Schacter[38] has conducted interesting studies on the effects of taste on eating by normal and obese adults. He reports that obese subjects are more responsive to the tastes of foods than are normal people. Several of his experiments represent analogues to nutritional studies with rats.[12]

At present there is no hypothesis that elaborates quantitatively how sensations of tastes and odors control eating. Recently, Le Magnen[25] and Jacobs & Sharma[20] have advanced some interesting ideas about how the sensations of taste and smell are involved in the control of food intake of animals. Jacobs & Sharma postulate that:

> ". . . physicochemical information from the diet feeds into two detector systems which can respond to signals from either taste or calories. Whether the CNS makes use of either set of signals in monitoring further intake is a function of the state of energy balance. The energy pool acts as a biasing system, assigning priority to taste when the animal is in deficit, and to calories when it is in balance or surfeit." (p. 1089)

Le Magnen[25] offers the notion that taste functions as a sensory cue in conditioned satiety and as an unconditioned stimulus for anticipatory metabolic reflexes. However, these hypotheses have not been critically evaluated by other laboratories.

It is apparent from this brief review that relatively little quantitative information exists about the effect of savoring on ingestion and on the development of food preferences in humans.

TASTE IN THE HUMAN NEWBORN

Studying the functions of taste in the human neonate offers several advantages:

1. Measurements of ingestion in response to variations in the gustatory qualities of nutrients can be obtained directly.
2. Olfactory and visual cues have not acquired the association with foods that they attain later when preferences and feeding habits develop.
3. The effects of experiential factors and socio-cultural attitudes on the acceptance of food are of less consequence than they are later in development.

Earlier studies have attempted to answer the question of whether or not the human neonate can taste. Taste receptors are developed before birth. Histological studies indicate that taste buds appear during the 2nd to 3rd fetal month.[14] Kussmaul[24] attempted to determine whether these receptors are functional at birth. He studied the facial expressions of infants after applying solutions of sucrose or quinine to the tongue, and concluded that the infant tasted sweet and bitter stimuli.

Table 18–1 presents a summary of several representative studies since Kussmaul's investigation that have attempted to answer the question of whether infants do taste. The results are equivocal; if the human newborn can taste, it is not clear from the findings in Table 18–1. These studies are difficult to interpret because of either the lack of quantitative measures of responses to taste stimuli or statistical analysis of the data. In our studies, the volume ingested by human infants is measured in response to variations in taste quality and intensity. Comparisons are made between the quantity of water and the quantity of stimulus solution consumed.

In a previous study,[7] we evaluated the infants' response to solutions of the common sugars: lactose, sucrose, glucose and fructose. Infants ingested larger quantities of sugar solution than of water. The sugar solutions were differentially ingested: sucrose was more preferred than glucose or lactose, and fructose was more preferred than glucose. As the concentration of the sugar solutions increased, the volume ingested was increased. We also noted an interaction between sex of the infants and the order of presentation of the water or sugar solution. Females displayed a greater preference for the sugar solutions when these were offered first.

The responses of the infants to the different sugars are replotted from this study and are presented in Figures 18–1—1–4. It should be noted that the interaction between the type of sugar and the concentration was not significant. Although the differences in these functions are suggestive, they are based on small and variable samples of responses.

TABLE 18–I

SUMMARY OF REPRESENTATIVE DATA ON THE INFANTS' RESPONSE TO TASTE STIMULI

INDICES:	*Sweet*	*Bitter*	*Salty*	*Sour*	*Age (days)*
Facial Expressions	Sucrose	Chloraquine	NaCl	Citric Acid	2
Aiyar and Agarwal (1969)		(Diluted to Saturated Solutions)			
	+	–	–	–	
Respiratory Responses &					
Movement of Fontanelles	Sucrose	Quinine	NaCl	Vinegar	1–14
Canestrini (1913)	0.06–0.15M	2%	0.34M	2–5%	
Eckstein (1927)	?	–	?	–	
Sucking Responses	Sucrose	Quinine	NaCl	Lemon Juice	¼–270
		(Diluted to Saturated Solutions)			
	+	–	–	–	
Pratt et al (1930)	Sucrose	Quinine	NaCl	Citric Acid	1–15
	0.49M	.25%	1.42M	1.11M	
	?	?	?	?	
Jensen (1932)	Glucose	NaCl	Acid	Not presented	
	0.33M	0.04–0.15M	Not presented		
	o	+	?		

Interpretation:
+ acceptance or like
– rejection or dislike
o indifference
? ambiguous

Validity Of The Taste Testing

Are the preferences we observed in our testing situation predictive of other parameters of normal feeding behavior? In an effort to assess this question, we compared the data obtained with our method with those obtained from normal feedings. The volume of nutrients consumed by infants during their regular hospital feedings is routinely re-

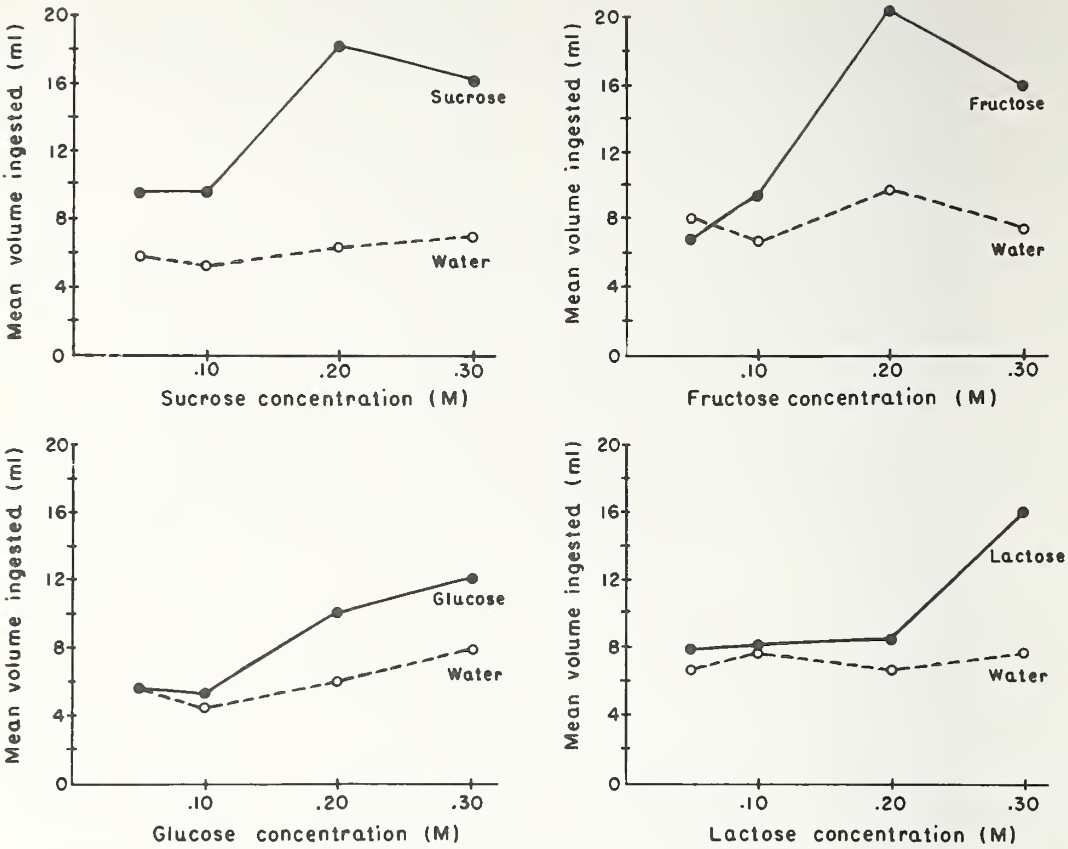

Figure 18–1–4. Mean volumes of solutions of sugars and water ingested by infants offered different concentrations of sugar solution. Fig. 1 (upper left) gives the response of sucrose, Fig. 2 (upper right), the response to fructose, Fig. 3 (lower right) the response to glucose, and Fig. 4 (lower left), the response to lactose (N=12).

corded in the hospital's records. Although these data are subject to several sources of error, the large number of measurements minimizes the possibility of consistent errors.

Two feeding regimens were used in the hospital during the course of our studies, and each of these regimens offers data comparable with those collected in our test situation. In one of these regimens an infant is offered one feeding of sterile water followed by two feedings of a 5% (0.28 M) glucose solution. These first three feedings occur at 12–15, 16–19, and 20–24 hours of age, respectively. Mean volume intakes (100 infants) at the first and second of these feedings are compared with the mean volume intakes of sugar solutions and water (192 infants) during our 3-minute testing situations (Fig. 18–5).

In another feeding regimen, the infants' first three feedings occur at 4–7, 8–11, and 12–15 hours following birth, respectively. The first two feedings offer sterile water and the third offers a low-density formula (Similac 13, Ross Laboratories). Mean intakes (100 infants) at the second and third feedings are compared with the volumes of water and formula ingested by 12 infants during our testing situation (Fig. 18–6).

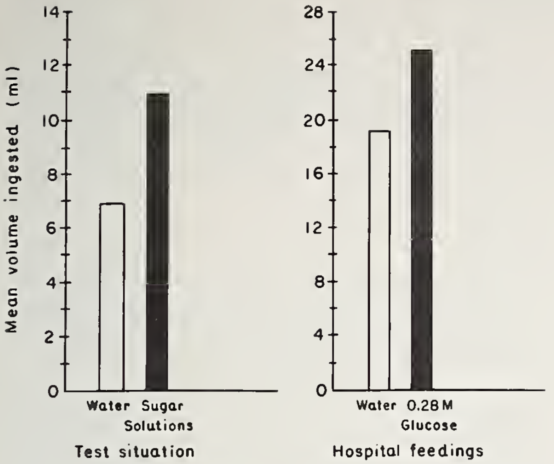

Figure 18–5. Mean volumes of solutions of sugar and water consumed by infants during a 3-minute testing situation (left side) and during routine hospital feedings (right side).

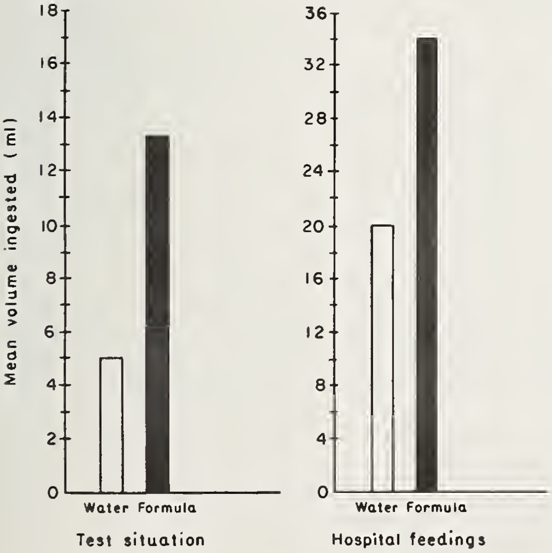

Figure 18–6. Mean volumes of formula and water ingested by infants during a 3-minute testing situation (left side) and during routine hospital feedings (right side).

In both normal feedings and the short-term test, infants demonstrated the same ingestive preferences. More of the sugar solution was consumed than was water in both the routine hospital feeding($t=4.75$, $df=99$, $p<.001$) and in the 3-minute testing situation ($t=7.30$, $df=191$, $p<.001$). See Figure 18–5. Likewise, preferences for formula over water were observed by both methods of measurement. (t feed$=9.48$, $df=99$, $p<.001$; t test $=4.39$, $df=11$, $p<.001$). See Figure 18–6.

Although the feeding data are confounded with variations in the age of the infants, their congruence with experimental results is suggestive. It appears that the short-term test is measuring variables that are relevant to normal feeding behavior.

Responses to sodium chloride, urea and citric acid.

Using the same testing paradigm as that used for the sugars, the observations were extended to other taste qualities viz. salty (NaC1), bitter (urea), and sour (citric acid). In addition, the interaction between the sex of the infant and the order of presentation observed in our initial study was tested here.

Subjects

102 male and 102 female infants, varying in age between 17 and 83 hours, were tested in their nursery at Pennsylvania Hospital. Full-term infants were tested if they were judged normal and healthy by a pediatrician, and were being bottle-fed. All of the babies tested had at least one feeding of a standard hospital formula (Similac 13, Ross Laboratories) before the testing.

METHODS

Procedure

Infants were tested between 11 AM and Noon, approximately midway between their regular 9:30 AM and 1:30 PM feedings. Babies were gently aroused from sleep and remained in their cribs, which were inclined forward slightly. Each baby was offered water for three minutes and a sapid solution for three minutes, with a two-minute rest period between presentations. The time periods were selected to minimize the contribution of postingestional factors upon ingestion[42] (e.g. calories, stomach distention and emptying, and intestinal absorption). As each bottle was offered, the nipple was stroked on the roof of the mouth for ten seconds to stimulate sucking. The order in which the solutions were offered was balanced. A preliminary evaluation of our method was discussed at the Third Symposium (pp. 372–374).

The fluids were presented in commercial nursing bottles with solid silicone rubber nipples. The nipples were fitted with a stainless steel standardized aperture and airbleed having an inner diameter of 0.686 mm.* The volume of each of the fluids consumed was measured to the nearest 0.5 ml.

The ingestion of solutions of sodium chloride, urea, and citric acid was measured. The concentrations of saline which were used ranged from 0.003 to 0.2 M, the concentrations of urea ranged from 0.03 to 0.18 M and solutions of citric acid varied from 0.001 to 0.012 M. Each solution was tested on six males and six females. In the case of sodium chloride, ten males and ten females were tested at concentrations of 0.006, 0.025, and 0.1 M.

Results

The differences between the volumes of sapid solutions and water that were consumed were analyzed by analyses of variance. The infants' responses to urea, citric acid, and salt are depicted in figures 18–7, 18–8, and 18–9, respectively.

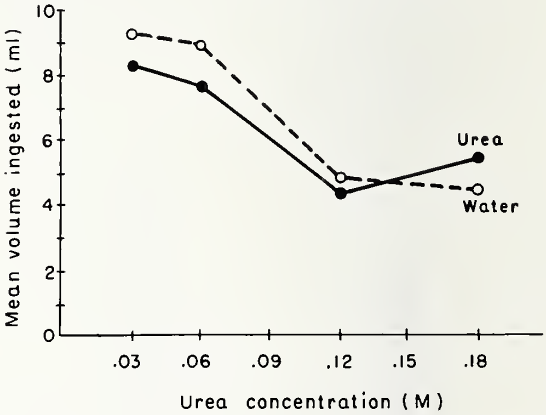

Figure 18–7. Mean volumes of solutions of urea and water ingested by infants who were offered different concentrations of urea (N=12).

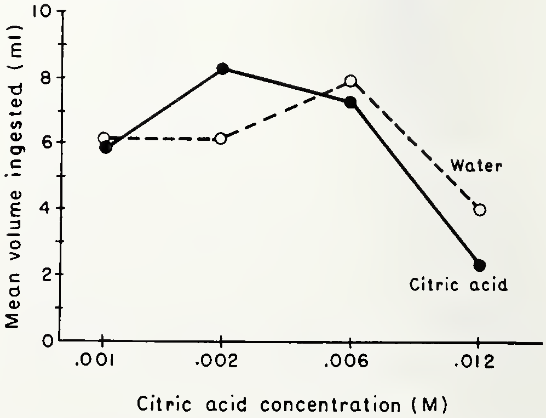

Figure 18–8. Mean volumes of solutions of citric acid and water ingested by infants who were offered different concentrations of citric acid (N=12).

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*Patent pending; for information and permission for use, write Dr. R. Kron, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104.

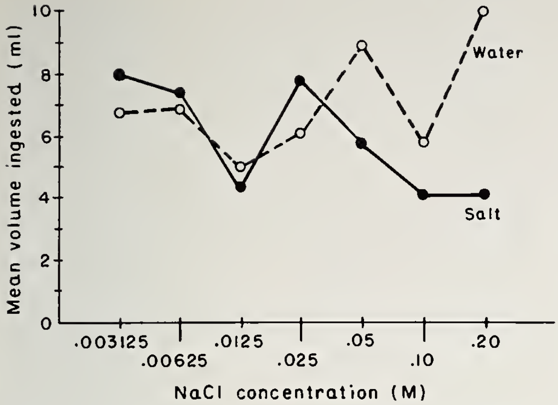

Figure 18–9. Mean volumes of saline solutions and water ingested by infants who were offered different concentrations of sodium chloride.

Human infants did not distinguish between bitter, sour, or salty solutions and water. The interaction between sex and order of presentation of the solutions observed with the sugars was not observed with any of these compounds. The infants' response to saline solution was interesting. Infants did not reduce their intake of the highest saline solution, but did tend to increase their consumption of water, although not significantly, when it followed the 0.2 M saline.

SUMMARY

The indifference of the human newborn to salty, sour, and bitter tasting solutions contrasts sharply with their distinct preference for sweets. Comparative studies of taste preferences generally indicate that sweet and salty solutions over a range of concentrations are preferred to water, whereas bitter and sour tasting solutions are responded to indifferently or are avoided.[15,26,32,33] There are some species exceptions to this generalization.[22]

The human infants' indifference to these compounds may be the consequence of one or all of the following: the concentrations were too low; the method used is not a sensitive index, or the infant perceives these taste stimuli but ingests them as readily as he does water. Each of these alternatives is briefly examined.

Detection and recognition thresholds, animal responses, and our data on the newborn's response to sucrose, urea, sodium chloride, and citric acid are summarized in Table 18–2. The concentrations of solution used in our studies are at least several times as large as the concentrations that evoke detection or recognition of these compounds in humans. In the case of saline and urea, the highest concentrations used were of the same order of magnitude as the concentrations of sugar that evoked a distinct preference. Also the increased water consumption in response to 0.2 M saline suggests that infants may differentiate it from water when it follows the ingestion of the salty solution. This hypothesis will be evaluated in subsequent experiments.

The concentrations at which indifference, preference, and aversion for these chemicals have been observed in subhuman studies are also tabulated. In the case of urea and citric acid, animals show aversion to the highest concentrations to which human infants were indifferent. The concentrations of saline solutions to which human infants were indifferent are preferred by animals.

Unfortunately, no other comparable data exists for human subjects to resolve the question of what are effective concentrations for differential ingestion of these solutions.

TABLE 18–II

COMPARISON OF CONCENTRATIONS (M) OF TASTE STIMULI EVOKING HUMAN AND ANIMAL RESPONSES

(References: 13, 15, 17, 19, 26, 32, 33, 34, 37)

Chemical and Taste Quality	*Human Thresholds*		*Animals' Responses*			*Infants' Responses*		
	Mean							
	detect.	*recog.*	*indifference*	*preference*	*aversion*	*indifference*	*preference*	*aversion*
Sucrose (sweet)	5mM	12mM	0.3–12mM	14mM–1.17M	1.61–2.34M	......	50–300mM	......
Citric Acid (sour)	0.4mM	0.7mM	0.1–10μM	......	0.1–10mM	1–12mM	......	......
Urea (bitter)	106mM	109mM	2–53mM	......	0.11–0.83M	30–180mM	......	......
Sodium Chloride (salty)	3mM	15mM	0.02–1.9mM	15mM–0.34M	0.36–0.51M	3–200mM	......	......

The third alternative is that the infant tastes salty, bitter, and sour solutions and accepts them as readily as water. Perhaps water is a poorly acceptable fluid to an infant, who may not be able to reduce the quantity of water consumed to levels lower than those obtained in our testing situation. Therefore, when reputedly "aversive" tasting stimuli are offered, a comparison between these two volumes may not reflect the infant's aversive response. We will test this post-hoc hypothesis in subsequent experiments.

Another possibility is that our expectations about the acceptability of salty, bitter and sour stimuli, which are based upon the data of subhuman studies of taste responses, are not indicative of the human's response to these compounds. Human indifference to these taste qualities may exist. There is anecdotal evidence, however, that some people have preferences for bitter-tasting beverages, for example, tonic water and coffee. Some people even like the sour taste of lemon juice and vinegar. Perhaps, hedonic responses to these tastes are learned, and are unlike the responses to sweet stimuli.

CONCLUSIONS

The human infant is able to distinguish water from solutions of sugar and prefers the latter. The preference for "sweets" appears very early in development and is relatively independent of experience. Infants were indifferent to solutions of citric acid, urea and sodium chloride. They may indeed be indifferent, and subsequent preferences or dislikes during development may be contingent upon experience with these tastes, and/or maturational factors.

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Chapter 19

TASTE-ELICITED TONGUE MOVEMENTS IN HUMAN NEWBORN INFANTS: AN APPROACH TO PALATABILITY

GEOFFREY H. NOWLIS

I am becoming convinced that innate, and perhaps acquired human preferences and aversions for various taste –sweetness, saltiness, sourness, and bitterness—have their origin in some relatively simple reflexes of the brainstem which can be traced phylogenetically as far as the primitive fishes. It is not my task to convince you of this; I have been asked to report on some of the very early results from a study of taste-elicited tongue movements in human newborn infants which I began recently in collaboration with Prof. William Kessen at Yale University. In order to provide a context for this study, however, I must give a brief sketch of some notions I have been developing concerning the fundamental importance of taste-elicited tongue reflex mechanisms in the vertebrate's decision to ingest or not to ingest potentially nutritive substances.

During chewing, lapping, and sucking, the tongue is quite active in positioning ingested materials with respect to the teeth, and with respect to the receptive zone for reflex swallowing,[8,17,31] toward the base of the tongue, in the pharynx. The proposition I should like to entertain here is that reflexive movements of rejection and acceptance on the part of the tongue represent the foundation of more complex acceptance and rejection behavior with respect to taste, and that the palatability of a given gustatory stimulus may be estimated on the basis of the extent to which that stimulus elicits impulses in the afferent limb of one tongue reflex (acceptance) in comparison with the other (rejection).

The form of the intake reflex in mammals, though expected to vary somewhat among species, is envisioned as a hollowing and curling up of the anterior portion of the tongue, accompanied by tongue retraction, to propel the contents of the oral cavity toward the receptive zone for the swallow reflex. The rejection reflex is envisioned as an arching up and outward (protrusion) of the posterior portion of the tongue which tends to propel mouth contents away from this swallowing trigger zone. A decision whether or not food should be directed

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further inward than the mouth must be made before it arrives back as far as this receptive zone for swallowing, for once it is there, swallowing must occur to protect the airway from the ingested material.

A further proposition is that one important way in which the hypothalamic regulatory centers express their control over food intake is by selectively biasing one reflex over the other, producing a predisposition toward intake in the case of hunger, and toward rejection in the case of satiation. Indeed, the notions concerning the importance of tongue reflexes as the basis of the behavioral categorization of substances as ingestible or non-ingestible, arose out of a consideration of the manner in which the hypothalamic nuclei might express their control over food intake. Rats with lesions of the ventromedial nuclei of the hypothalamus, in addition to hyperphagia and obesity, manifest a paradoxical "finickiness" with respect to alterations in the palatability of the food offered them.[6] Offered a palatable diet, they will overeat; offered the same diet adulterated with, say, quinine, at a level which is still quite tolerable to intact animals, they will drastically reduce their intake, and may stop eating altogether. It seemed that if the palatability of a given substance were basically a function of a balance between two brainstem reflexes, one of rejection, and one of acceptance, then the behavior of the organism without rostral control over such reflexes would be rather strictly determined by the properties of the stimulus activating the reflexes. Thus, a gustatory stimulus which activates an acceptance reflex to a greater extent than a rejection reflex should be ingested as long as ingestion remains possible. Similarly, a gustatory stimulus which elicits a rejection reflex to a greater extent than it does an acceptance reflex should determine quite strictly that the substance not be ingested. If such reflexes, in the intact organism, are under the tonic control of the hypothalamus, they might be facilitated or inhibited according to information from blood chemistry, or even olfactory input, and hence the behavior of ingestion or rejection would be modulated according to the state of the organism, thus not being so strictly determined by the properties of the peripheral stimulus.

These considerations constitute a neurological version of an interpretation that psychologists have made of the behavior of obese human beings[19,30] and of animals made obese by hypothalamic lesions:[32] the decision whether or not to ingest food seems in these individuals to be more dependent on external cues (such as taste) than on internal cues (such as those chemical cues in the blood which presumably correlate with number of hours since the last meal). Consideration of the afferent and efferent innervation of the tongue will illustrate the rationale for the present, somewhat mechanistic formulation, in that it suggests a more precise formulation of the postulated reflexes, and the manner in which they may combine to produce the typical acceptance and rejection behavior with respect to the various basic gustatory stimuli. This, in turn, will lead us to the possibility that we can ask a newborn infant whether he does or does not like a given tasty fluid simply by observing the movements of the baby's tongue as he samples the fluid by sucking.

The anatomical essentials for the argument are schematized in Figure 19–1. The anterior and posterior portions of the mammalian tongue are distinct mus-

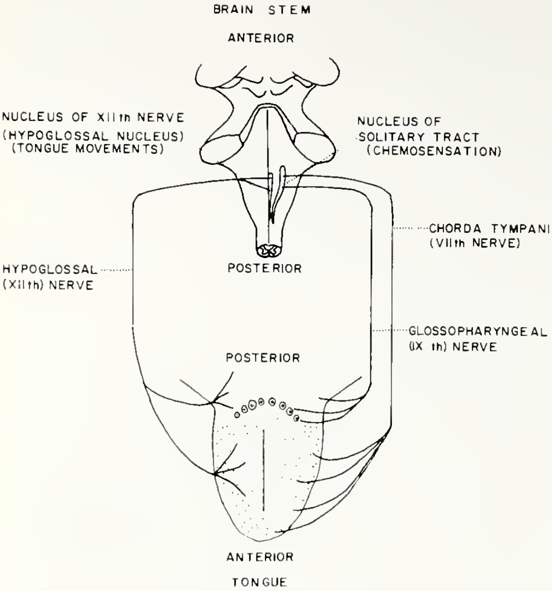
BRAIN STEM
ANTERIOR
NUCLEUS OF XIIth NERVE (HYPOGLOSSAL NUCLEUS) (TONGUE MOVEMENTS)
NUCLEUS OF SOLITARY TRACT (CHEMOSENSATION)
CHORDA TYMPANI (VIIth NERVE)
HYPOGLOSSAL (XIIth) NERVE
POSTERIOR
GLOSSOPHARYNGEAL (IX th) NERVE
POSTERIOR
ANTERIOR
TONGUE

Figure 19–1. Schematization of structures which may be involved in taste-elicited tongue reflexes.

cular groups, and the anterior portion is considered by some anatomists[7] to be a mammalian innovation. Separate branches of the hypoglossal nerve account for a relatively independent activation of these muscle groups from the motor neurons of the hypoglossal nucleus. The afferent innervation of the taste buds on these two portions of the tongue is even more distinct; the fungiform papillae of the anterior portion of the tongue send gustatory information to the rostral portion of the nucleus of the solitary tract via the seventh cranial nerve, while the circumvallate and foliate papillae of the posterior portion send gustatory information to more caudal portions of this nucleus via the ninth cranial nerve.[1] Although each of these nerves, along with the tongue area each supplies, is responsive to some degree to each of the four basic tastes, there appear to be systematic differences in their relative sensitivity.[24,33] Thus, according to classical accounts,[33] the tip of the tongue is most sensitive to sweet, and further back, but still within the receptive zone of the seventh nerve, the edges are most sensitive to salt; still further back in this zone, sour is more strongly represented. Once the transition is made to the area of the ninth nerve, bitterness is predominant. Thus, both in terms of the anterior-posterior distribution within the seventh nerve receptive field, and in terms of the more distinct transition from seventh to ninth nerve zones, the input to the nucleus of the solitary tract, following the topographic organization of the tongue, is biased rostrally in favor of sweet and salt, which are preferred in moderately concentrated acqueous solutions to plain water by most species, and caudally in favor of bitter and sour, to which water is generally preferred (see below). It should be noted, however, that the responses of the secondary gustatory neurons in the rostral (seventh nerve relevant) portion of the nucleus of the solitary tract[15] have not yet been the subject of the type of study which would allow a generalization to the effect that, *within* this portion of this nucleus, there is a rostral to caudal progression of sensitivity to sweet, salt, sour, and bitter.

This classical picture of the localization of taste on the human tongue has recently been questioned,[9,10] the higher sensitivity of the posterior portions of the oral cavity to bitter and sour being attributed to taste buds on the palate. However, since the responsiveness of these extra-lingual taste buds is blocked by anesthetization of the ninth and tenth cranial nerves in the palate,[9] whose destinations are the more caudal portions of the nucleus of the solitary tract, these results are consistent with the model proposed here. I might add that, in pilot studies with three decerebrate cats, I have electrically stimulated the palate in the area of these taste buds and produced movements of the tongue which correspond to my idea of a rejection movement: an arching up and protrusion of the posterior tongue. Although the stimulation in these instances was as likely of somatosensory as of gustatory receptors, these observations lend some support to the notion that sensory stimulation in this part of the mouth can evoke tongue movements of the appropriate form.

Directly ventral and medial to this sensory nucleus of the solitary tract lies the hypoglossal nucleus, responsible for motor innervation of the tongue.[5] Simple reflex connections between topographically analogous portions of these parallel nuclei (either at this level or more rostrally, for example, at the level of the recently discovered[20] pontine gustatory nucleus), could readily innervate the postulated reflexes in such a manner that the intake reflex be more strongly evoked by stimuli which preferentially stimulate the anterior tongue receptors, the rejection reflex more strongly by stimuli which preferentially stimulate the posterior tongue receptors. It does seem that, within the hypoglossal nucleus, rostral stimulation leads to tongue retraction, while more caudal stimulation evokes protrusion.[13] Furthermore, as discussed below, the ratio of activity in the ninth nerve (rejection) to that in the seventh nerve (intake), from very strong solutions of sweet and salty substances is relatively higher than for weaker solutions, providing a basis for rejections of these substances which, at lower concentrations, preferentially stimulate the seventh nerve, and are generally accepted.

If this picture of the reflex basis of gustatory preference and aversion is valid, the activity in the seventh and ninth nerves in response to a given gustatory stimulus might be used for a reasonably accurate prediction of the palatability of that stimulus. To the extent that gustatory impulses are sent to the rostral portions of the solitary tract, intake activity should be evoked. To the extent that gustatory impulses are sent to the caudal portions of the nucleus of the solitary tract, rejection activity should be evoked. Thus, the activity evoked by a given gustatory stimulus in the seventh nerve (weighted, perhaps, according to the anterior-posterior location within the receptive zone for this particular taste), minus the activity evoked by this stimulus in the ninth nerve, should yield a quantitative estimate of the relative palatability of the stimulus. Such a simple subtraction for stimuli across a range of concentrations of a given basic taste should yeild a preference-aversion curve for that taste. I shall illustrate this procedure below.

Such preference-aversion curves are available for many different species. I have included two sets here, from the work of Prof. Carl Pfaffman and his associates.[22,23] Figure 19–2 is for the albino rat; Figure 19–3 is for the squirrel monkey, to illustrate some of the remarkable interspecies similarities. Species differences do, of course, exist, and my suspicion is that some of these differences might be accounted for on the basis of species differences in the sensitivities of the two nerves. The perference-aversion curves represent the percentage of the organism's total fluid consumption attributable to consumption of a flavored fluid, when offered in a two-bottle preference test[26] with plain water in one continuously available bottle, and some concentration of the flavored solution in a second con-

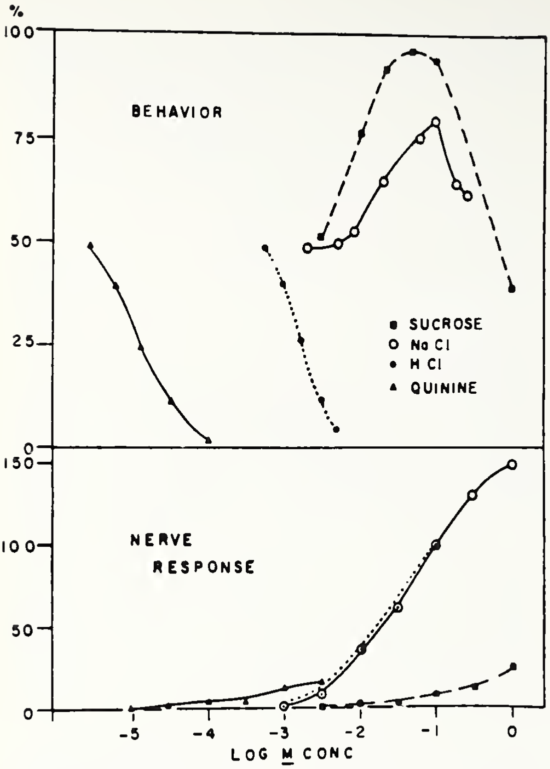

Figure 19–2. Composite graph of preference-aversion functions and integrated whole chorda tympani responses in the rat. The upper half shows percentage intake of the taste solution as a function of concentration. The lower half shows chorda tympani responses in arbitrary units. (from Pfaffmann, 1960).

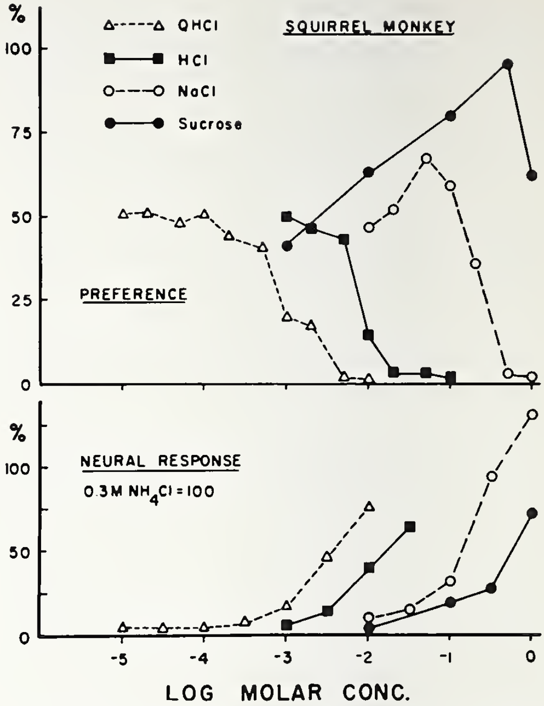

Figure 19–3. As in Figure 2, but for squirrel monkey. (from Pfaffmann, 1965).

tinuously available bottle. Thus, a value greater than fifty percent indicates that more than half of the organism's intake in this choice situation is intake of the flavored substance, taken to indicate a preference. Values less than fifty percent are taken to indicate an aversion plain water is ingested to a greater extent than the flavored solution.

The neural activity (integrated whole nerve activity from the chorda tympani branch of the seventh nerve) is also plotted in the lower half of Figure 19–2 and of Figure 19–3. It is interesting that, whereas the neural activity is a monotonic function of concentration of each of the four basic tastes, the palatability is not necessarily a monotonic function of concentration. It is this aspect of the palatability curves which helped lead to the current formulation, involving a *positive* contribution of seventh nerve activity to palatability, and a *negative* contribution of ninth nerve activity to palatability. Figure 19–4 shows a comparison of integrated whole nerve activity from the seventh nerve (chorda tympani) and ninth nerve (glossopharyngeal) in the rat, taken from studies by Pfaffman, Fisher, and Frank.[24] Let us concentrate for a moment on the curves for sodium chloride.

Notice that although the activity in the ninth nerve begins to rise at a higher concentration than does that in the seventh nerve, the former rises at a steeper rate than does the latter. Thus, at lower concentrations of salt, there is more seventh nerve activity, while at

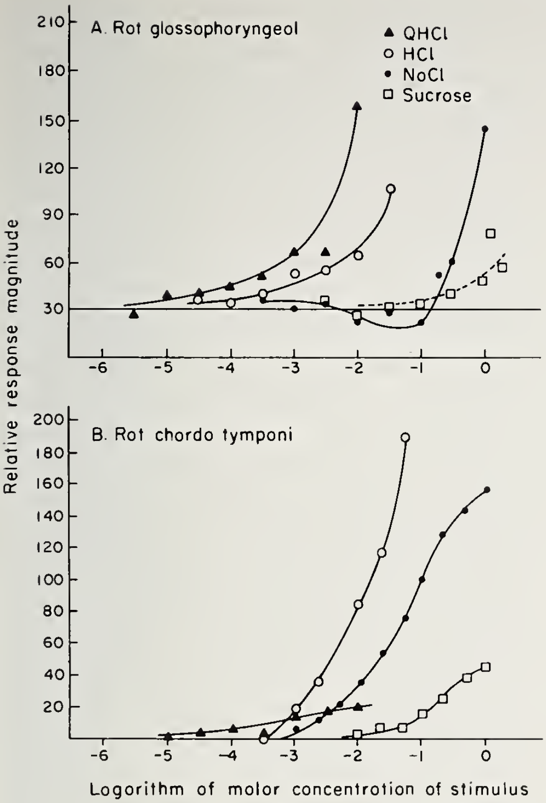

Figure 19–4. Composite graph showing integrated whole nerve responses in arbitrary units from rat glossopharyngeal nerve (top half) and rat chorda tympani (lower half), as a function of concentration of solutions of quinine hydrochloride (bitter), hydrochloric acid (sour), sodium chloride (salty), and sucrose (sweet). (from Pfaffmann, Fisher, and Frank, 1967).

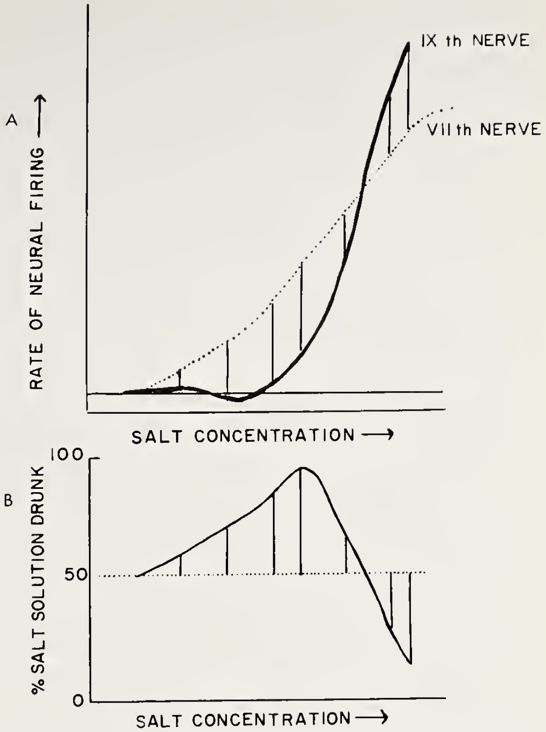

Figure 19–5. Schematization of derivation of rat preference-aversion function for sodium chloride from neural activity functions.

A. Integrated whole nerve responses in seventh and ninth nerves as a function of concentration of sodium chloride, replotted from data of Pfaffmann et al (1967) from Figure 4.

B. "Preference-aversion" function for sodium chloride, derived by subtracting ninth nerve activity from seventh nerve activity at the points indicated by vertical lines.

higher concentrations, there is more ninth nerve activity. Figure 19–5 illustrates the simple procedure of subtracting the curve of ninth nerve activity to salt from that of the seventh nerve activity. When the difference between the two curves is positive (seventh greater than ninth) this difference is plotted above the fifty percent, or neutral palatability level, as an increment in palatability. When the difference is negative, it is plotted below the fifty percent level, as a decrement in palatability. The resultant theoretical curve bears a remarkable resemblance to the sodium chloride preference-aversion curve for the rat shown in Figure 19–2. Similar curves, corresponding quite well to the empirical palatability curves, can be generated for the other three basic tastes. Look, for example, at the two neural response curves for quinine hydrochloride (bitter) in Figure 19–4. The threshold for ninth nerve activity is lower, and the activity increases more rapidly as a function of concentration, than in the case of seventh nerve activity in response to quinine hydrochloride. Thus, subtracting the ninth nerve curve from the seventh nerve curve, an increasingly negative difference results with increasing concentration, producing a monotonic downward sloping curve from the neutral palatability level, that is, a monotonic decrease in palatability with increasing concentration of quinine—just as in Figure 19–2.

Indeed, this simple method can produce theoretical curves which correspond very closely to the empirical palatability curves if one proceeds by assuming either that the arbitrary units of neural response magnitude in Figure 19–4 should be re-scaled logarithmically, or that the contribution of the seventh nerve for a particular taste should be weighted more positively, the further anterior that given taste is represented on the tongue, providing an educated guess as to how far rostrally this activity is represented in the nucleus of the solitary tract. It should be noted, however, that this correspondence is not to be construed as proof that the activity in these two nerves accounts for the preference behavior observed. It is simply offered to demonstrate that enough information is available from a comparison of the two nerves, as a result of their differential sensitivity, to be used as the potential basis for the observed behavior. A fuller theoretical account of palatability related behavior may have to await recordings of the tenth nerve response to gustatory stimulation. This nerve innervates taste buds of the oropharynx (and chemoreceptors in a number of digestive organs), and sends impulses to a still more caudal portion of the nucleus of the solitary tract.[5]

An interesting and encouraging line of evidence related to this reflex model of food intake is a consideration of comparative vertebrate modes of food intake and comparative vertebrate neuroanatomy. I have not yet fully pursued this line of evidence, and such a discussion is too lengthy to be appropriate here. One example, however, from C. Judson Herrick, should not be omitted. Fishes have taste buds on the tongue, and often also distributed on the outside of the body, in the skin or on whisker-like barbels.[11] Herrick[11] has shown that an intake reflex is elicited by gustatory stimulation of the taste buds on the outer surface of the body in several species of fishes, which consists of a turning of the body and head toward the stimulus, and an opening and closing of the mouth onto the source of stimulation. The tongue is apparently used primarily for rejection. The taste buds on the outside of the body are all innervated by the seventh nerve—there is no structure in fishes corresponding to the anterior portion of the mammalian tongue. The taste buds on the tongue of fishes are all innervated by the ninth nerve. Herrick has worked out potential anatomical reflex connections between the seventh nerve portion of the nucleus of the solitary tract and the appropriate motor nuclei for the intake response described in fishes. In the mammal, there are no external taste buds, and the seventh nerve innervates instead the taste buds on the anterior portion of the tongue.

We have begun three different lines of research based on this model of palatability and food intake. One line attempts to demonstrate the existence, behaviorally and electrophysiologically, of the postulated reflexes, and the influence, if any, of hypothalamic stimulation on them, in chronically maintained decerebrate cats. Another line attempts to pursue the encephalization of the preference-aversion behavior by comparing the effects on palatability of seventh or ninth nerve section in intact rats,[21] with the effects of such nerve section in rats with lesions in the parts of the neocortex, thalamus, and striatum which are concerned with sensory or motor aspects of tongue and mouth. All of these projects are in somewhat preliminary stages at this moment, but I should like to describe some of our findings to date in a third line of research, dealing with palatability and tongue movements in infants.

The infant studies take off from the recent evidence[2,14,28] that lapping movements of the tongue are among the most important components of the sucking response in human newborn infants. Sameroff[27,29] has developed a pressure-transducing nipple which permits independent measurement of this lapping response and the negative pressure, or suction component of the sucking reflex. We have modified this nipple considerably (Figure 19–6) to allow a further analysis of the lapping component of the sucking response: independent measurement of the pressure exerted by the anterior and posterior portions of the infant's tongue against the medical grade silicone rubber nipple. If the proposed reflex model of palatability is applicable to the human infant, the pressure from the anterior portion of the tongue should be greater when a highly palatable substance is delivered via the nipple. Similarly, the pressure from the posterior portion of the tongue should be greater when an unpalatable substance is offered. Our hope is to use this set of measurements to make a derived measurement of palatability in the infant.

The peculiar shape of the nipple is dictated by several constraints. First, in order to ensure independent compression of the two fluid-filled compartments of the nipple (connected to Statham P23A pressure transducers), the rest of the nipple had to be relatively rigid. Second, in order to measure the pressure from the posterior tongue, the nipple had to be long enough to protrude rather deeply into the infant's mouth. Figure 19–7 shows a highly schematized drawing of the head and oral cavity of a newborn infant, derived from sketches and castings from the oral cavity of a cadaver. The arrow indicates the approximate point of transition between the anterior and posterior portions of the ton-

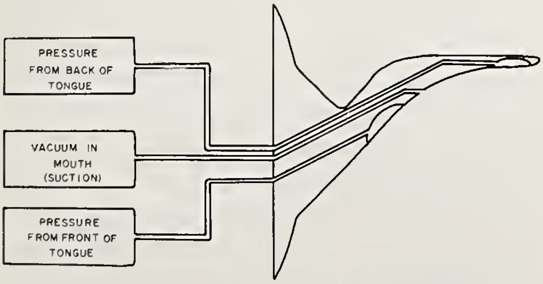
PRESSURE FROM BACK OF TONGUE
VACUUM IN MOUTH (SUCTION)
PRESSURE FROM FRONT OF TONGUE

Figure 19–6. Diagram of recording nipple.

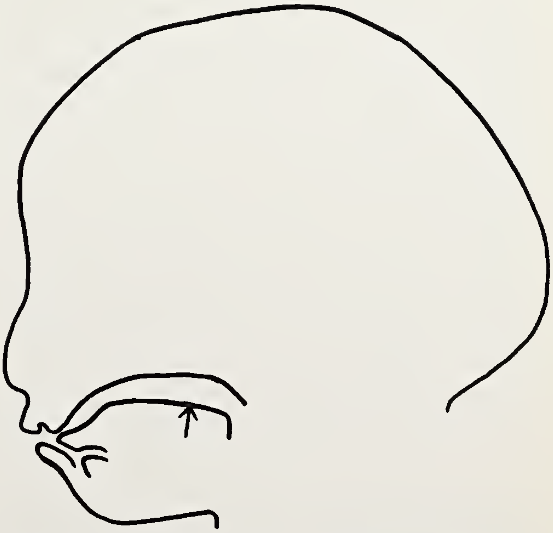

Figure 19–7. Schematized mid-saggital view of newborn infant's head, showing tongue in oral cavity. Recording nipple apposes palate and protrudes into mouth as far as arrow.

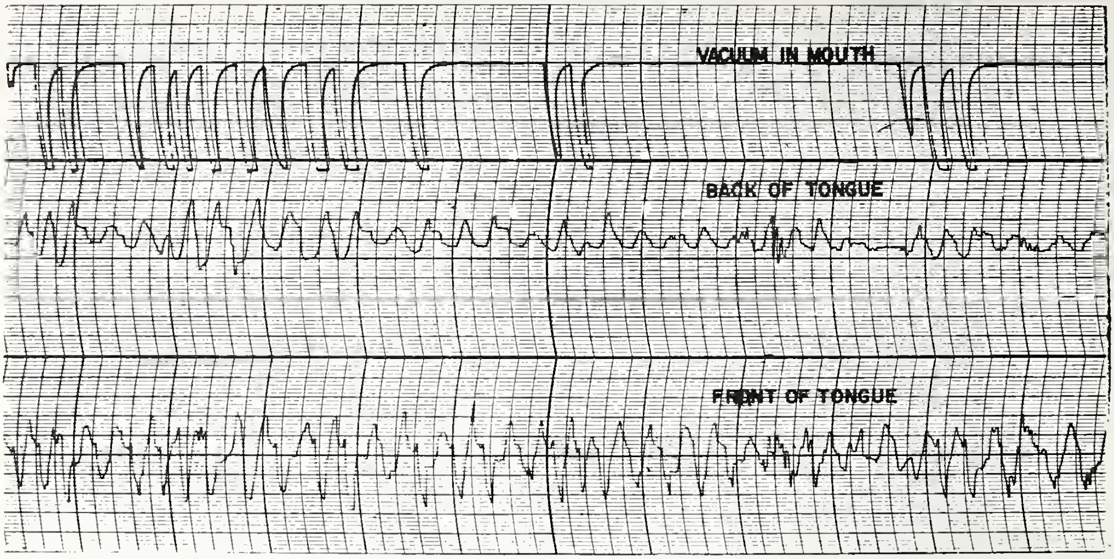

Figure 19–8. Sample of pressure variations from front and back of tongue and vacuum in mouth during newborn's sucking while being fed standard hospital formula. Two horizontal divisions equal one second. Vertical axis is not calibrated.

gue of the newborn, beyond which the nipple must intrude. Third, in order not to elicit gagging and the accompanying rejection movements of the tongue with the tactile stimulation from the rigid nipple itself, it had to be made as unobtrusive as possible, thus accounting for the tapering tip and the dorsal arch of the nipple, designed to conform to the shape of the palate. Parallel to the tube for measuring suction is a second tube, not illustrated in Figure 19–6, through which fluids may be delivered.

A sample of the data recorded with an earlier version of the nipple, arranged to deliver formula to the infant, is illustrated in Figure 19–8. Notice that the rhythmic recordings from the front and back of the tongue are out of phase with one another, indicating that the two pressure-sensitive areas of the nipple are recording events occurring at different points in the temporal sequence of the sucking response. It is also evident that the channel representing the vacuum created in the infant's mouth with each suck is not always active when a suck is indicated by tongue pressure measurements. Close examination of the occasions accompanied by this phenomenon suggests that the infant is still sucking, but that the lips fail to seal perfectly around the nipple, thus not allowing a vaccuum to be created in the mouth.

The population with which we have been working consists of healthy term infants in the first three days of life. Our procedure consists of allowing the infant to suck at the nipple, with two or three milliliters of a sterilized taste solution available for ingestion. Thus, with a given infant, a number of different solutions can be offered in a thirty to forty-five minute period without ingestion of appreciable amounts of solute. A rigorous experimental design has not yet been imposed upon our procedure, but our pilot studies do seem to allow a few generalizations from the phenomena which we see consistently among the sixty infants we have observed in perfecting our technique.

1. If dextrose or sucrose is offered, the amplitude of the response from the anterior tongue increases with successive sucks of a sucking bout until a maximum amplitude is reached, and sustained over a number of sucks. This is followed, still within the same sucking bout, by a decrease in amplitude in the anterior pressure with each of a few final sucks antecedent to the termination of the bout. During this sequence, the amplitude of the posterior tongue response remains fairly constant.

2. The peak amplutide of the anterior tongue response for a given infant is greater for strong than for weak concentrations of dextrose and of sucrose. The implication is that the amplitude of the anterior tongue response is a positive function of concentration of each of the two sugars so far employed. This generalization is somewhat limited, however, by the fact that we were restricted in our pilot studies to two percent (0.058 M) and four percent (0.117 M) sucrose, and five percent (0.277 M) and ten percent (0.555 M) dextrose. We are initiating a study employing four equivalent molar concentrations each of sucrose, dextrose, lactose, and fructose.

3. The peak amplitude of the anterior tongue response to two percent (0.058 M) sucrose was generally as great as, or greater than the peak amplitude of the anterior tongue response to ten percent (0.555 M) dextrose, while the peak amplitude of the anterior tongue response to four percent (0.117 M) was still greater. The implication is that a given concentration of sucrose elicits a stronger anterior tongue pressure than does an equivalent concentration of dextrose (although a little extrapolation is necessary to make the statement in this form, since equivalent concentrations were not employed). This generalization is consistent with the observation[18] that sucrose is estimated as sweeter than dextrose at equivalent concentrations by adult subjects.

4. When a 10^{-4} M solution of quinine hydrochloride is offered, after a few sucks the bout of rhythmic sucking is disrupted. This is often followed by isolated movements of the posterior tongue which exert a sustained pressure against the nipple for one to two seconds. This movement is not accompanied by pressure from the anterior tongue. We have not yet explored many different concentrations of unpleasant tasting stimuli. One reason is that the current version of the nipple is most sensitive to those movements of the posterior tongue which are directed upward, toward the palate, whereas the gross movements of rejection which we can feel as we hold the nipple in the infant's mouth are definitely directed outward, tending to push the nipple out of the mouth. This particular movement registers only rather feebly and inconsistently with the current nipple, which is now being modified to better record the rejection movements. However, by producing mixtures of quinine and sucrose, we have been able to elicit a rhythmic sucking response in some infants in which the posterior tongue participates well and the anterior tongue participates very little, if at all, according to our pressure measurements.

5. In the very few infants to whom physiological saline (0.154 M NaCl) has been offered, some individuals showed a pattern of response resembling a mild version of the response ro 10^{-4} M

quinine hydrochloride; others, offered NaCl concentrations one-quarter to one-tenth as strong, showed signs either of mild rejection or of non-vigorous acceptance. One possible reason for the inconsistent response to these moderate concentrations of NaCl is that the salivary sodium content from one newborn infant to another is quite variable,[12,25] ranging from around 0.01 to around 0.07 molar. Within three to six months, this value drops, in healthy individuals, to a fairly stable value of about 0.008M, which is maintained into adulthood. An implication of this is that the taste receptors of the infant's mouth may be adapted to a variety of sodium concentrations. It has been shown[3,4,16] that concentrations of NaCl at about the value at which an adult's tongue is experimentally adapted are estimated as tasteless, while concentrations below this adapting concentration are estimated as more strongly *bitter* (or sometimes sour), the lower the concentration of salt. Such a phenomenon considerably complicates the interpretation of responses to solutions of sodium chloride; our intention is to include measurement of salivary sodium content in further stuides of the infant's response to salt. The possibility exists that the receptors in the circumvallate papillae in the posterior tongue are adapted at still another value, determined by the secretions of Von Ebner's glands which bathe these receptors, rather than by the salivary sodium, further complicating the behavioral implications of a given experimental salt concentration, or plain water, for that matter.

In short, our preliminary results indicate that the newborn infant is capable of responding differentially to quantitative and qualitative differences among gustatory stimuli, and that the nature of these different responses seems so far to be consistent, if not yet completely, with the proposed reflex model of palatability. We are thus somewhat optimistic that our measurements of tongue pressures will serve as a basis for quantitative estimates of newborn infants' preferences and aversions for gustatory stimuli.

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DISCUSSION OF CHAPTERS 17, 18 AND 19

GASSER: I would like to point out that in studying the development of the extramedullary part of the facial nerve in human and subhuman primate embryos, one finds the chorda tympani to be the first branch to form, followed closely by the greater petrosal nerve that probably also contains taste fibers.[1,2] These two branches are first evident in 4.8 to 6.5

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[1] Gasser, R.F. 1967 The development of the facial nerve in man. Annals of Otology, Rhinology and Laryngology, *76*:37–56.

[2] Gasser, R.F. and A.G. Hendrickx 1967 The development of the facial nerve in baboon embryos (*Papio sp.*). Journal of Comparative Neurology, *129*:203–218.

mm human embryos with a fertilization age of approximately 4 weeks. The branches are identifiable long before any of the muscular branches on the face. Just shortly before this period, closure of the rostralmost portion of the neural tube took place. The diencephalic and telencephalic parts of the prosencephalon can only be distinguished by the position of the optic vesicle.

The nerves carrying taste fibers and the geniculate ganglion can be identified before the cerebral vesicles that will form cortex. At the time the cerebral vesicles are evident, the chorda tympani has joined with the lingual part of the mandibular nerve where the submandibular ganglion is forming.

STEINER: The anatomical basis of taste appears to be ontogenically very old. Taste also seems to be a phylogenically ancient sense. May I add that, as all other chemoceptive senses, taste is also able to function in a wet medium. This is not the place to go into details but I think we have enough evidence from the strong connection between salivation and taste to state that what the aquatic vertebrate (e.g. fish) can taste on his body surface, terrestic forms (among them human beings) can taste only in their oral cavity.

KAWAMURA: There are many reports on brain mechanisms for taste perception by means of an ablation technique. Removal of the cortical taste area and removal of the thalamus strongly affects taste preference behavior in mature animals. Your results indicate strong contributions of the lower brain to taste preference. Do you think that discrepancy between your results in human infants and previous reports in animals may be attributed to species difference, or to the effects of maturation?

STEINER: I can imagine that both factors you mentioned, species differences and maturation, can explain the discrepancy if such discrepancy exists. I don't know whether the disturbances in taste-preference-behaviour due to removal of relevant cortical and thalamic areas are persistent or transient. I would expect that a preference behaviour in a mature animal can be severely disturbed by ablation experiments but, knowing from my findings about discriminatory ability based on functions due to ponto-medullar structures, I would expect a restitution of the preference behaviour.

KAWAMURA: A second question. As you know, a single taste fiber or a single taste neuron in the solitary nucleus may respond to different taste qualities. At the present time, the pattern theory of taste representation is more generally accepted than the specific theory. If so, when you apply one modality of taste stimulation to the tongue, the one taste neuron in the solitary nucleus may respond and send the impulse to the facial and the trigeminal motor nuclei. When you stimulate with a different modality of taste, the same taste neuron in nucleus solitarius may send impulses to these motor nuclei.

However, your studies show obvious differences in the response of the facial and the mandible muscles when you applied different kinds of taste stimuli to the tongue. Do you have any ideas about the background brain mechanisms of these discriminatory phenomena?

STEINER: I have really very little speculation to add to my phenomenological approach but I can imagine that such discriminatory processes can be based on combinations between firing neurons and silent neurons. If we accept specificity of single neurons in the solitary nucleus it is easy, of course, to explain the different facial reactions.

KAWAMURA: Does a change in the concentration of the tastant result in a different facial expression?

STEINER: In the framework of this study we worked only with one set of concentration of the tastants.

SUMI: Could you give us your ideas about the neural organization which is capable of discriminating such differences of taste. The response which you show is so stereotyped that I am comparing it with swallow. Is it possibly analogous to the "swallowing center"?

STEINER: I can only speculate about neural organizations.

I can not name them otherwise than "innate circuits" which are responsible for discrimination. I see parallelism between the taste discrimination ability and the discriminations between non-noxious and noxious stimuli achieved by the nociceptive system. The nociceptive system and the defense reflexes linked by this system express in a very similar way that type of discrimination which I named "good-for-me" or "bad-for-me".

SUMI: We should be careful about ascribing or interpreting purpose to particular responses of an infant to particular tastants. For instance, when you get a response in a baby to sweet, you interpreted that he relaxes and desires some more. When you give a bitter tastant, you note that he hesitates and wants to get no more. How could you know that he desires or that he hesitates?

STEINER: I am absolutely aware of the possibility that reactions, especially *human reaction observed by human beings* can be a matter of interpretation. I would like to turn your attention, again, to my concluding remarks in which I said that I attach less importance to the particular appearance of one facial expression or the other, and I think that the most striking point is that the ancient part of the CNS is equipped with a discriminatory ability inducing motor reactions which reflects acceptance and aversion respectively. From the fact that photographed facial reactions of babies mean basically the same thing to everyone, you can see the validity of this non-verbal expression of hedonic values.

BOSMA: Dr. Steiner, apropos of the local autonomic response to the application of sour tastant to the infant's tongue, you described transient flushing of the skin. Was there also flushing of the oral mucosa at lips and tongue?

STEINER: I did not record these phenomena.

THACH: Did you employ a salty tastant?

STEINER: The application of a salty tastant did not elicit typical or easily classifiable responses. These responses require our further study.

KENNY: As I was watching Dr. Steiner place strong chemicals on tongues of infants with cold pipettes I couldn't help but wonder what role he considered for the fifth nerve in his gustofacial response?

STEINER: I would like to remind you of my methodology. I used disposable plastic pipettes. The chemicals rinsed on the tongue were of room temperature. I am aware of the fact that most techniques of taste stimulation have to include tactile and thermal factors. I think that taste stimuli especially above threshold are dominant and mask the simultaneous tactile and thermal input. Only the use of a temperature controlled, constant pressure and constant flux chamber can insure elimination of the attendant non-gustatory stimuli. Such a chamber is appropriate for animal experiments, preferably with general anaesthesia. But not for humans, and especially not for human infants.

The most carefully designed experimental set-ups, such as the microstimulation in von Bekesy's[1] experiments or the procedure demonstrated here by Doctors Weiffenbach and Thach,[2] are able to reduce the tactile component in the application of tastants.

As for my own results I can only point to the clear difference between reactions induced by tasteless water and sapid chemicals, all applied by the same technique.

BOSMA: Dr. Nowlis approaches the mouth and pharynx as a region having a variety of sensory relevant functions, including maintainance of position at the mouth and about the pharyngeal and laryngeal airway, and participation in tidal respiration and its various adaptations, as well as the oral and pharyngeal aspects of feeding. In earlier symposia, I have designated this as a "portal area". The optimal definition of the area's functions, to which we should aspire, is in terms of their sensory elicitation and guidance.

Different arrays, or complexes, of local sensations are involved in egressive, compared with ingressive, portal area performances. In preliminary experiments in cats, Takagi, Irwin and I[3] found that discrete stimuli applied to the mucosa of the epiglottis and adjacent parts of the lower pharynx elicited modulations of the expiratory phase of tidal respiration, while stimulation in the epipharynx differentially elicited modulations of inspiration.

By subjective experience the epiglottis and adjacent parts of the lower pharynx are also the site of detection of acidic gastric contents incident to emesis. Perhaps this chemosensation is at the taste buds found in this region. This could be an aspect of defense of the lower airway against penetration of regurgitated gastric contents. In some analogy, acidic emesis materiel is also detected subjectively in the epipharynx. And taste buds are found, also, on the upper aspect of the soft palate.

JOHNSON: I share Dr. Bosma's enthusiasm for this work. And I also am glad that the airway was mentioned because we are indeed considering the entrance to the airway as well. Particularly in reference to newborn work, the matters of swallowing and breathing arise.

When you are talking about swallowing, I don't see how you can possibly eliminate the stimulus from being applied to the entrance to the airway, at the aditus of the larynx. In the newborn, this region of the airway is readily challenged, with resultant transient or prolonged apnoea, depending on the age and state of the animal. I wonder if any of your observations looked at cardiovascular or respiratory effects. Because they certainly will limit the apparent length of action of the stimulus and, thus, the interpretation of the results. Did you observe apnoea when challenging the airway as well at the mouth and pharynx in these experiments?

STEINER: No, never.

NOWLIS: I should like to comment briefly on the possible relevance of the emesis to which Bosma refers and the rejection reflex I've postulated, and of the swallowing reflex to the lingual intake reflex. I have had occasion to observe a few decerebrate cats which I have maintained chronically to observe the recovery of rhythmic oral movements after surgery. These observations, along with the classical studies of Miller and Sherrington[6] and of Bard and Macht[1,5] present the following picture.

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[1] Von Bekesy, G.: The effect of adaptation on the taste threshold observed with a semiautomatic gustometer. *J. Gen. Physiol.* 48:481–488, 1965.

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Within minutes after decerebration, as soon as the anesthetic wears off, the entire swallowing reflex is very easily elicitable, either with electrical or with fluid stimulation around the root of the tongue in the pharynx.[6] At this stage of recovery, stimulation of the anterior tongue with a piece of food elicits no response. However, the swallowing reflex itself does entail movements of the tongue[6,4] such that on each elicited swallow, the tongue protrudes slightly, the anterior portion hollows and curls, and then retracts just in time to avoid getting caught between the jaws, which now close tightly as an inevitable part of the swallow reflex.

Within two weeks, if a bolus of food, for example a piece of liver small enough for swallowing, is placed on the anterior tongue, it appears as if the sequence of swallowing can be initiated from this region. The tongue protrudes slightly, hollows and curls, and propels the bolus toward the back of the oral cavity, where it may elicit the rest of the swallow reflex by stimulating the pharyngeal receptive field for this reflex. Several such anterior tongue movements, rhythmically repeated and alternated with jaw closure, are elicited if the initial ones are not sufficient to position the bolus correctly for swallowing.

Interestingly enough, if the bolus is fairly large, and cannot readily be swallowed, once the tongue has positioned it back toward the receptive zone for swallowing, the posterior tongue arches up and pushes the bolus back toward the front of the mouth, where it may restimulate the anterior tongue intake movement, again followed by the closure of the jaw which would follow this anterior tongue action were the entire swallow sequence activated. It thus seems possible that the lingually elicited tongue and jaw movements represent a truncated version of the swallow reflex, and that rhythmic oral activities such as mastication might derive from repeated incomplete elicitations of the reflex, which continue until the rhythmic activity has altered the properties of the mouth contents sufficiently to allow a complete swallow.

There is a sense, then, in which the recovery of sensitivity in the oral cavity following decerebration follows a caudal to rostral time course, at least insofar as stimulation can elicit movements: stimulation in the pharynx elicits the swallow reflex immediately after decerebration; stimulation of the anterior tongue does not elicit tongue movements until a few days to two weeks following decerebration. These observations would seem to fit nicely with Dr. Bosma's[2] observations in the Third Symposium concerning the caudal to rostral development of function in the

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[1] Bard, P. and Macht, M.B. The behaviour of chronically decerebrate cats. In: Wolstenholme, G.E.W., (Ed.) *Ciba Foundation Symposium on the Neurological Basis of Behavior.* Boston: Little, Brown, 1958, 55–75.

[2] Bosma, J.F. Form and function in the infant's mouth and pharynx. In: Bosma, J.F., (Ed.) *Third Symposium on Oral Sensation and Perception*, Springfield, Ill., Thomas, 1972, 3–29.

[3] Brizzee, K.R. Effect of localized brainstem lesions and supradiaphragmatic vagotomy on irradiation emesis in the monkey. *Amer. J. Physiol.* 187:567–570, 1956.

[4] Doty, R.W., and Bosma, J.F. An electromyographic analysis of reflex deglutition. *J. Neurophysiol.*, 19:44–60, 1956.

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portal area of the alimentary tract. Furthermore, after one to two weeks recovery from decerebration, if an object which cannot be fully ingested, such as a finger, is placed in the mouth of the cat, rhythmic motions of the anterior tongue, alternated with jaw closures, are elicited. To my finger, which has explored the configuration of sucking movements in many human infants, these movements of the chronic decerebrate cat's mouth resemble suckle movements in remarkable detail. Again, these anteriorly elicited movements are not elicitable during the first few days after decerebration.

Bard and Macht[1,5] have observed that rejection movements of the tongue from gustatory stimulation are recovered in the chronic decerebrate cat, such that liver chunks adulterated with strong solutions of various tastes, although initially propelled inward by the anterior tongue, are immediately ejected, presumably by movements of the posterior tongue. If the intake tongue movements are viewed, as I have suggested, as a maturationally dependent independence of the oral phase of the pharyngeal swallow, it would seem plausible to speculate that such taste-elicited rejection movements represent a similarly independent elicitation of the lingual phase of retch or emesis. The caudal portions of the nucleus of the solitary tract have been implicated as part of the afferent pathway for emesis,[3] and this observation fits nicely with the notion of chemically elicited tongue movements of rejection mediated by the caudal portions of this nucleus.

MISTRETTA: I am pleased that Dr. Bosma has mentioned extra-tongue taste buds, which are present not only in newborns but in adults of some species. Taste buds are present on the soft palate,[1,2] epiglottis[2] and in the nasoincisor duct[3] of the rat. They are also found on the soft palate of rabbits[2] and on the epiglottis of the cat and dog.[4] As I mentioned in the Third Symposium, the study of these extra-lingual taste buds has been neglected.

I would like to address a question to Dr. Nowlis. Denervation studies[3,5,6] have demonstrated that it is very difficult to alter taste preference-aversion curves in the rat by interrupting the afferent taste input to the central nervous system. Admittedly, it is virtually impossible to denervate all taste buds in the rat tongue and oropharynx. However, the results of the attempted denervation studies seem to be at odds with some of your ideas on the relation between ingestive behavior and sensory input. Would you please comment?

NOWLIS: It is good that you have mentioned these studies. I left this issue out of my talk in order to be brief. I have begun, in collaboration with J. Jay Braun, a series of studies which I hope will speak to the issue. In the first study,[7] we have removed the insular cortex which constitutes the cortical

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projection[1] of the thalamic relay nucleus for taste, and, using a one-bottle preference test, have obtained preference-aversion curves which differ very little from those of intact rats. One difference, however, might be anticipated in the next study: in the case of rats with cortical lesions, section of the ninth cranial nerve may be anticipated to eliminate aversions, while seventh nerve section may eliminate positive preferences. That this is not the case in the otherwise intact rat was clearly shown by Pfaffmann,[8] who observed no substantial alteration in the preference-aversion curves unless both nerves were sectioned, and in this case, a simple flattening of the curves was observed.

The rationale, very briefly, by which one might expect the appropriate denervation to alter preference or aversion in animals with gustatory cortical lesions, and not in animals with intact cortex, is imagined as follows. It is consistent with some views of the vertebrate nervous system[5] that the gustatory pathways involved in sending information from the nucleus of the solitary tract to the thalamo-cortical projections, bypass the interneurons believed responsible for the postulated reflexes. Since each nerve carries information about the concentration of each of the four basic tastes, a given concentration of a gustatory stimulus could, by learning, be associated with a given predisposition toward rejection or acceptance (this latter information arriving at the neocortex via phylogenetically older pathways). Information, then, from either nerve about absolute concentration, should be able to produce normal preference behavior in the rat with intact cortex. The neocortically guided preference-aversion behavior, then, is imagined as responsive to, but not compelled by, the reflex organization of the older parts of the nervous system. That a learned gustatory averion[3] can easily be acquired in one[6] or a few[9] trials to a specific concentration of a gustatory stimulus has recently been demonstrated.

In the event that the appropriate nerve section does not eliminate preferences of aversions in the rats with lesions of the gustatory neocortex, one may reasonably push the issue one or two more empirical steps before abandoning the model. Bard and Macht[2,4] have shown that decerebrated cats, maintained chronically after surgery, regain, after about two weeks, the function of rejecting foods adulterated with the four basic tastes at about the same concentrations as produce rejection in normal intact cats. Thus at least some palatability-related behavior is integrated at the level of the brainstem. It is possible that only at this level are preferences and aversions so purely and immediately a function of reflexes that the appropriate denervations produce alterations in behavior. The encephalization of such reflexes must certainly be a complicated matter. Even if the reflex control of ingestion does not emerge to be as simple as I am imagining it here, the studies implicit in pursuing such a model will hopefully shed some light on the nature of taste-elicited behavior in organisms with interruption of the central pathways involved in sensory and motor representation of the tongue.

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MALLER: A comment about units of concentration. The literature on taste suffers from a lack of uniformity in specifying concentrations. Molarity is the unit of concentration which should be employed in specifying concentration. The use of percentage as a unit of concentration has resulted in considerable confusion because it can be expressed in a number of ways. A standardization of the unit of concentration in terms of *molarity* would facilitate comparisons of studies.[1]

The question I have is directed to Dr. Steiner. I am sure you are aware of the general problem in obtaining good inter-observer agreement about what emotional state is represented by facial expressions in humans.[2]

Have you any data on observer agreement about the facial expression evoked by a particular taste stimulus?

STEINER: Yes. The correlation between the observers judgment is high.

MALLER: I hope you will give considerable attention to the methodological problems involved in using facial expression as an index of taste responsivity. Have you done a double-blind study or do you plan to do one?

STEINER: The present study was not designed in a double-blind paradigm. Future behavioral studies will be in that design.

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Chapter 20

LINGUAL LIPASE

MARGIT HAMOSH AND ROBERT O. SCOW

INTRODUCTION

It is generally accepted that pancreatic lipase plays a key role in the digestion of dietary lipid.[21] However, in the rat pancreatic lipase activity is low at birth and does not increase until after weaning.[33,37] Thus, at a time when more than 70% of the calories are derived from milk lipid, it would appear that the mechanism for lipid digestion is not yet developed.

Milk of several species has lipase activity[7,22,23,30] suggesting the possibility that lipase present in milk could be active in the digestion of lipid in the newborn. Therefore, we analyzed stomach contents of suckling rats and found high concentrations of free fatty acids (FFA),[15,19] indicating that milk lipids were hydrolyzed in the stomach. Further studies, however, showed that the lipolytic activity of rat milk was too low to account for the hydrolysis found in the stomach.[16] Gastric mucosa of adult rats has been shown to have slight lipolytic activity,[2,3] but this activity is specific for medium chain triglycerides and has very little effect on long chain triglycerides,[3] the major constituent of rat milk. With the milk, pancreas and gastric mucosa thus excluded as the source of the lipolytic activity in the gastric contents of the suckling rat, we decided to test the secretory tissues of the oral cavity for lipase activity.[17]

METHODS

Adult female and 3-6 day old suckling rats were used in these experiments. The animals were killed by decapitation and the tissues were removed, chilled and homogenized in cold glucose-free Tyrode's solution.

Lipolytic activity in tissue homogenates, saliva and gastric contents was measured by using as substrate triglyceride in chylomicrons, milk or corn oil.[17] Chylomicrons containing triglyceride labeled with ^{3}H-glycerol and ^{14}C-fatty acids were isolated from thoracic duct chyle after tube-feeding rats corn oil containing ^{14}C-palmitic acid and triolein labeled with ^{3}H-glycerol.[18,35] Use of doubly labeled triglycerides markedly increases the sensitivity of the assay, while at the same time allowing rapid characterization of the reaction products.

The lipase assay system contained 50 μmoles buffer (Tris-maleate, pH 5.0–6.0; Tris-HCl, pH 6.5–8.0; or citrate Na_2HPO_4, pH 3.2–7.4), 25 mg bovine plasma albumin, 2 μmoles chylomicron-triglyceride, 0.5–20 mg tissue homogenate and water in a final volume of 0.5 ml.[17] Incubation was at 37°C in a Dubnoff shaking bath for 1–120 min.

When the substrate was nonlabeled triglyceride (in rat milk, bovine milk or corn oil) a slightly modified assay system was used.[17]

Triglyceride and the products of enzymatic activity (diglyceride, monoglyceride, FFA and glycerol) were analyzed by thin layer[39] and column chromatography.[5,10,12] Radioactivity in the samples was measured by liquid scintillation spectrometry. FFA,[8] glycerol[9] and triglyceride[29] were measured by methods described previously.[17]

TABLE 20–I

HYDROLYSIS OF CHYLOMICRON-TRIGLYCERIDE BY HOMOGENATES OF STOMACH AND ORAL TISSUES OF SUCKLING AND ADULT RATS

Tissue	*No. of expts.*	*FFA produced*	*Optimal pH*	*Inhibition by 0.5 M NaCl*
		μEq/g per hr		%
Suckling Rats				
Stomach contents	3	124 ± 10	5.4	0
Stomach	2	20 ± 6	5.0	0
wall	2	31 ×± 13	8.0	0
Submandibular & sublingual glands	2	0	–	–
Anterior half of tongue	2	12 ± 2	5.4	–
White tissue near circumvallate papilla	2	2,700 ± 900	4.5–5.4	0
Adult Rats				
Submandibular gland	1	6	7.4	–
Sublingual gland	1	16	7.4	–
Parotid gland	1	4	7.4	–
Anterior half of tongue	2	19 ± 1.0	5.4	–
White tissue near circumvallate papilla	2	11,800 ± 500	4.9–5.4	0
White glandular tissue anterior to circumvallate papilla	4	50,100 ± 10,000	4.9–5.4	0
Soft palate	3	410 ± 200	5.4	–
Anterior oropharyngeal wall	3	1,640 ± 330	5.4	–
Lateral oropharyngeal gland	3	1,480 ± 300	5.4	–

Values are means ± SE. (Hamosh and Scow, ref. 17)

RESULTS

Lipolytic activity of homogenates of stomach and oral secretory tissues

Hydrolysis of chylomicron-triglyceride by tissue homogenates is shown in Table 20–I. In suckling rats the stomach contents had lipolytic activity, releasing FFA from chylomicron-triglyceride at a rate of 124 μmoles/g per μ hr. The stomach wall, however, had only very low lipolytic activity. The large salivary glands had no lipolytic activity and the anterior part of the tongue only traces of activity, whereas the posterior part of the tongue had very high lipolytic activity.

Similar results were obtained with preparations from adult rats. The salivary glands and anterior half of the tongue had only traces of activity, whereas the back of the tongue had very high activity. Survey experiments localized the activity to the white glandular tissue anterior to the single large circumvallate papilla (Fig. 20–1). Homogenates of this tissue in adult rats released from chylomicron-triglyceride 11,800 μmoles FFA/g per hr. and that in suckling rats 2,700 μmoles/g per hr. Sections of adult rat tongue examined grossly and microscopically showed that the glandular tissue in the region of the circumvallate papilla consisted of serous and mucous glands (Fig. 20–1), and that the secretory ducts of the serous glands opened into the trough of the circumvallate papilla and into the folliate papillae (Fig. 20–2).[13,14] Homogenates of serous glands had extremely high lipolytic activity, releasing 50–90,000 μmoles of FFA/g per hr whereas mucous glands from the same area had low activity, releasing 800 μmoles of FFA/g per hr. Lipolytic activity was also found in the soft palate, anterior oropharyngeal wall and the lateral oral pharyngeal glands, but the level of activity was only 1–3% of that in the lingual serous glands (Table 20–I and Fig. 20–1).

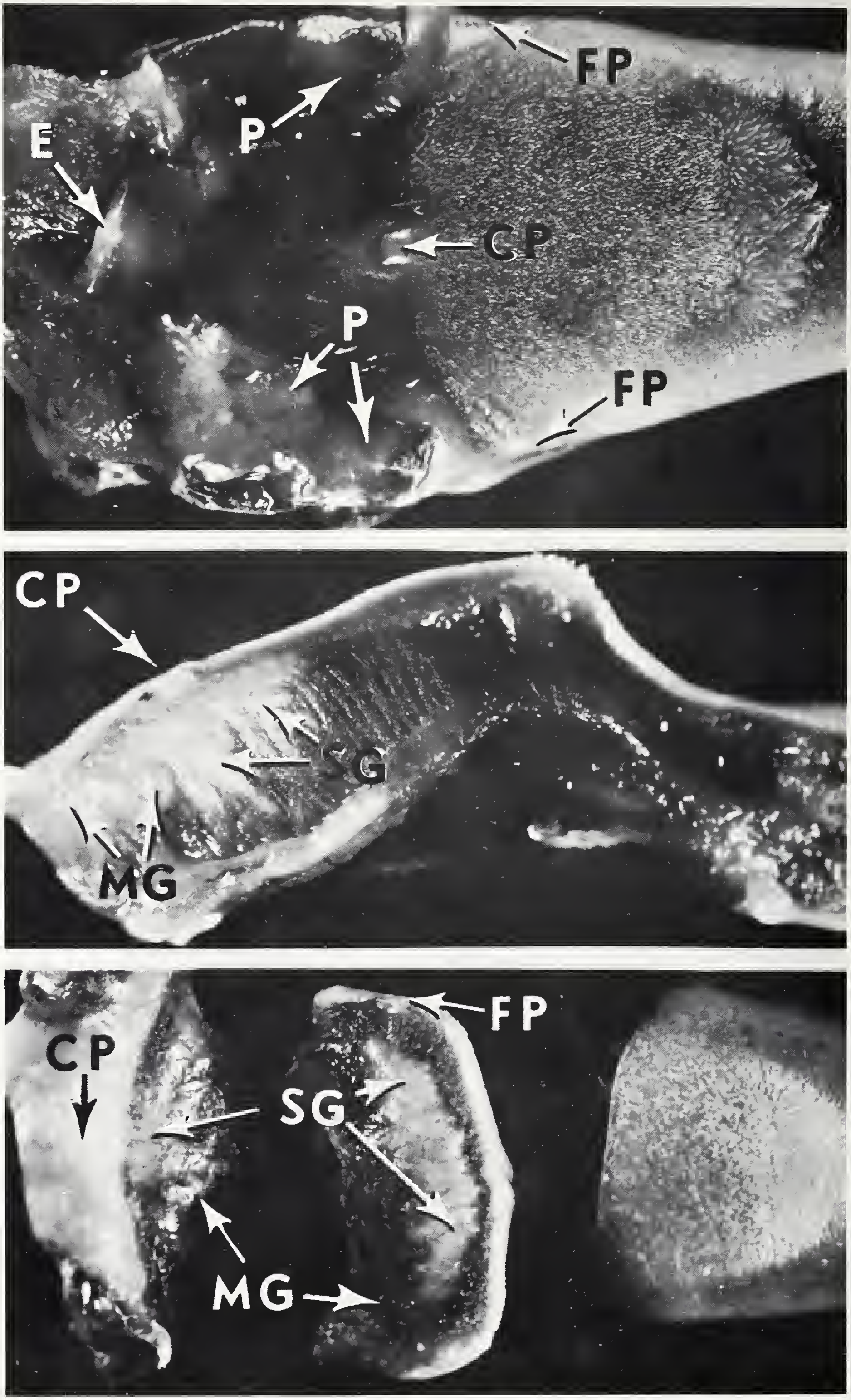
Nature of the lipolytic activity in tongue and gastric contents

The liploytic activity in homogenates of tongue and gastric contents of suckling rats and tongue of adult rats had very similar pH optima, in the range of 4.5–5.4 (Fig. 20–3). The activity in homogenates of tongue was about 25 times higher than that present in the stomach contents in suckling rats. The low pH optimum and lack of inhibition by 0.5 M NaCl indicated that the activity was not that of lipoprotein lipase.[31,32]

Homogenates of lingual glandular tissue from suckling rats hydrolyzed chylomicron-triglyceride and milk triglyceride to diglyceride, monoglyceride and FFA; diglyceride accounted for 85% of the partial glycerides formed during the first 5 minutes of incubation and 70% at the end of one hour (Fig. 20–4). Partial glycerides and FFA were the main reaction products even after one hour of incubation; less than 5% of the triglyceride was completely hydrolyzed to glycerol and FFA. Milk triglyceride and chylomicron-triglyceride were hydrolyzed at similar rates during the first 15 minutes of incubation, about 1 mmole/g per hr. Similar reaction products were obtained with homogenates of gastric contents of suckling rats and with lingual glandular tissue of adult rats, the main reaction products being FFA and partial glycerides (diglyceride accounting for 70–85%, and monoglyceride, 10–30%) and less than 5% glycerol.

Intragastric hydrolysis of triglyceride and the role of lingual lipase

About 10% of the lipid present in the stomach of suckling rats was in the form of FFA.[15,19] Tables 20–II and 20–III show the results of experiments designed to determine whether intragastric lipolysis occurs in the adult rat. Milk triglyceride was readily hydrolyzed in the stomach of adult rats fed a mixture of bovine milk and cream; 12% of the triglyceride was hydrolyzed within 10 minutes to diglycerides and FFA (Table II). An additional 14% was hydrolyzed *in vitro* during incubation of stomach contents in albumin solution at pH 5.4 for 60 minutes. The pH of the stomach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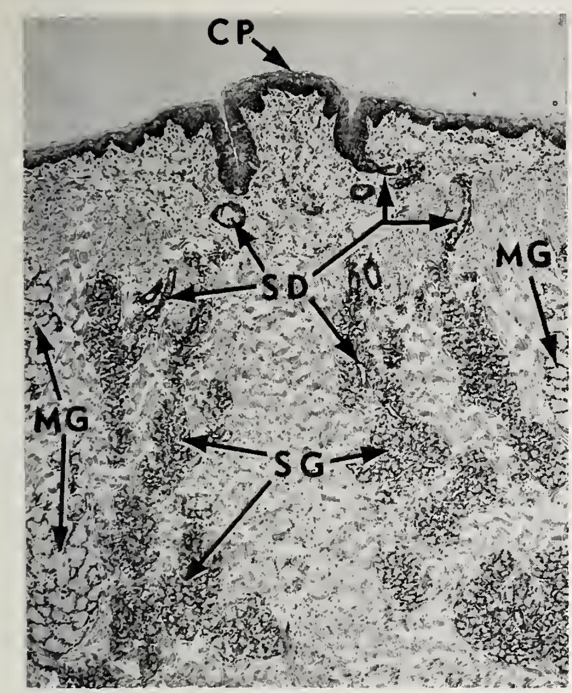

Figure 20–2. Rat tongue: Section through the circumvallate papilla (CP) showing serous glands (SG) below the papilla and mucous glands (MG) on each side. The secretory ducts (SD) of the serous glands open into the furrows of the circumvallate papilla. X 40. (From Hand (14)).

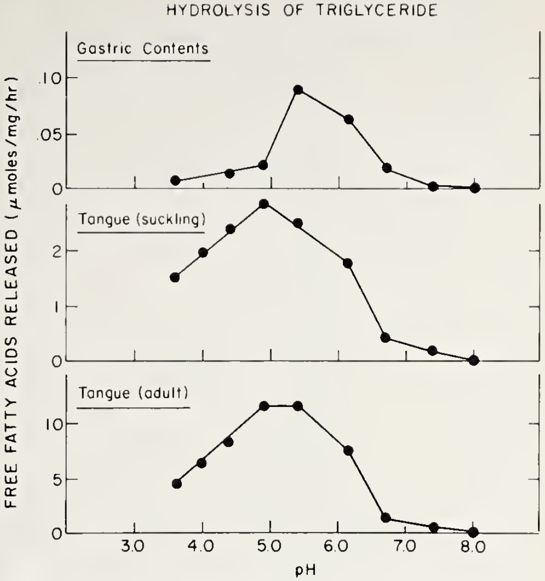

Figure 20–3. Effect of pH on hydrolysis of chylomicron-triglyceride by homogenates of gastric contents (suckling) and tongue (suckling and adult). Each assay tube contained 2 μmoles of doubly labeled triglyceride, 0.5 ml of 5% albumin in 0.1 M citrate-Na_2HPO_4 buffer solution and either 20 mg of stomach contents of suckling rats, 2.0 mg of white tissue from near the circumvallate papilla of suckling rat tongue, or 0.5 mg of white glandular tissue from near the circumvallate papilla of adult rat tongue. The values are means of 3 experiments. (Hamosh and Scow, ref. 17.)

≪ Figure 20–1. Rat tongue: (a) Top view showing circumvallate papilla (CP), foliate papillae (FP), lateral oropharyngeal gland (P), and epiglottis (E). (b) Sagittal section through the circumvallate papilla showing serous glands (SG) below and anterior to the papilla, and mucous glands (MG) posterior to the papilla. (c) Cross section through the foliate papillae and anterior to the circumvallate papilla, showing serous glands anterior and antero-lateral to the circumvallate papilla and near the foliate papillae. Mucous glands are present below, lateral and posterior to the serous glands. (From Hamosh and Scow (17)).

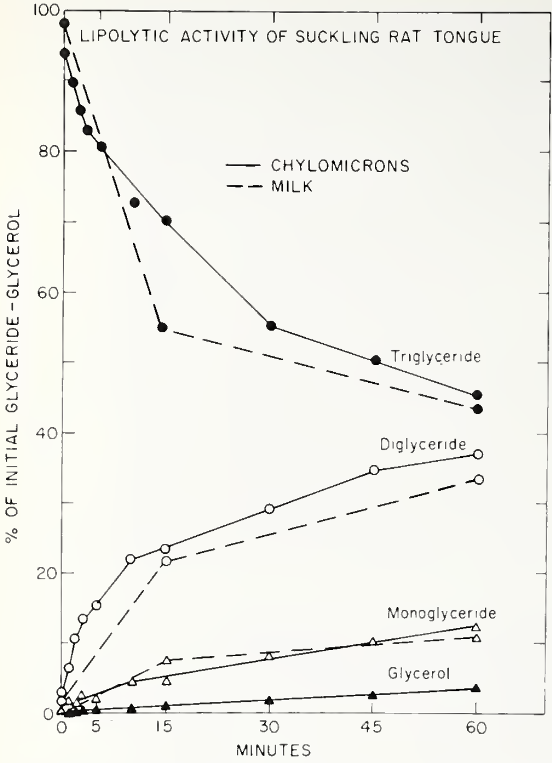

Figure 20–4. Suckling rat tongue: Hydrolysis of chylomicron-triglyceride and rat milk-triglyceride by homogenates of white tissue from near the circumvallate papilla.
Chylomicron-triglyceride: Each tube contained 2 μmoles of doubly labeled triglyceride, 2 mg of tissue homogenate, and 0.5 ml of 5% albumin in 0.1 M citrate-Na_2HPO_4 buffer solution at pH 5.4. The mixture was incubated at 37°C. The values are means of 3 experiments.
Milk-triglyceride: Each tube contained 6 μmoles of triglyceride, 10 mg of tissue homogenate, and 0.5 ml of 5% albumin in 0.1 M citrate-Na_2HPO_4 buffer solution at pH 5.4. The mixture was incubated at 37°C. The values are means of 3 experiments. (Hamosh and Scow, ref. 17.)

contents (4.5–5.5) and the nature of the reaction products (diglyceride and FFA) suggested that lingual serous gland lipase could be active in the intragastric digestion of triglyceride. The experiments described in Table III support this assumption. When saliva was excluded from the stomach through a cannula inserted into the esophagus, only traces of FFA were found in the stomach (0.8% of total fatty acids) 30 minutes after tube feeding the milk-cream mixture, and there was only a slight increase in FFA when the gastric contents were incubated for 1 hr *in vitro*. In the normal controls, 8.5% of the fatty acids were present as FFA 30 minutes after ingestion of the mixture of milk and cream, and incubation of the gastric contents increased the FFA content 300% in 1 hr.

Corn oil fed in a purified diet was also hydrolyzed to diglyceride and FFA in the stomach. About 20% of the triglyceride was hydrolyzed within 10 minutes and 29% within 20 minutes (Table IV). The pH in the stomach contents of the rats fed the corn oil diet was 4.3–5.0.

TABLE II
HYDROLYSIS OF BOVINE MILK-TRIGLYCERIDE IN THE STOMACH OF ADULT RATS

					Lipid Composition				
Specimen	No. of expts.	pH	Total fatty acids		Tri-glyceride	Di-glyceride	Mono-glyceride	FFA	Amount of triglyceride hydrolyzed
			μEq	μEq/g wet	% of glyceride-glycerol			% of total fatty acids	μmoles
Test meal	1	7.0	-	495	94.8	4.9	0.2	0.5	-
Gastric contents									
After feeding 10 min	4	4.5–5.5	1,330 ± 310	543 ± 45	83.3 ± 1.7	16.4 ± 1.4	0.4 ± 0.1	3.4 ± 0.1	51
Incubated 15 min	2	5.4	-	-	76.8 ± 2.8	22.5 ± 2.5	0.7 ± 0.1	6.1 ± 1.0	80
Incubated 60 min	2	5.4	-	-	70.0 ± 2.0	29.0 ± 2.0	0.9 ± 0.1	9.1 ± 0.8	110

Rats were fed for 2 days a mixture of bovine milk and cream. They were then fasted overnight and fed the milk-cream mixture *ad libitum* for 10 min. The gastric contents were then removed and aliquots were taken for analysis and incubation with 7.5% albumin solution at pH 5.4 and 38°. Values are means ± SE. (Hamosh and Scow, ref. 17)

TABLE 20–III

EFFECT OF EXCLUSION OF SALIVA ON THE HYDROLYSIS OF BOVINE MILK-TRIGLYCERIDE IN THE STOMACH OF ADULT RATS[1]

Group	*No. of expts.*	*pH of gastric contents*	*FFA formed in gastric contents*[2] *In vivo*[3] *(35 min)*	*In vitro*[4] *(60 min)*
			% of total fatty acids	
Control	5	4.5–5.5	8.5 ± 0.9	32.6 ± 3.4
Cannulated	4	4.5–5.5	0.8 ± 0.02	3.3 ± 1.1

[1] Flow of saliva to the stomach was diverted by inserting a cannula into the cervical portion of the esophagus 1–3 hr before experiment. The cannulated rats were tube-fed and the control rats were fed *ad libitum* 6 ml of a mixture of bovine milk and cream containing 10–12% triglyceride by weight. All rats were fasted overnight prior to experiment. Values are means ± SE.

[2] FFA comprised 0.3% of the fatty acids in the milk-cream mixture.

[3] *In vivo:* The gastric contents were analyzed 30–40 min after feeding.

[4] *In vitro:* An aliquot of the stomach contents was incubated in 7.5% albumin solution at pH 5.4 for 60 min. (Hamosh and Scow, ref. 17)

CONCLUSIONS

These studies describe a potent lipolytic activity present in the lingual serous glands of the suckling and adult rat.[17] The lipase hydrolyzes triglyceride from a variety of sources (corn oil, milk or chylomicrons) to mainly diglyceride and FFA at a pH optimum of 4.5–5.4. Activity with similar characteristics was present in gastric contents of suckling and adult rats and in saliva collected through an esophageal cannula. There was very little activity in the stomach when saliva was excluded. The pH of gastric contents was 4.5–5.5 in fed rats, both suckling and adult, and in tube-fed rats in which saliva was excluded from the stomach. The activity was also present in glandular tissue from the soft palate, oral pharyngeal wall and oral pharyngeal glands.

The secretory ducts of lingual serous glands (of von Ebner) open into the furrows of the circumvallate and folliate papillae.[4,13,14,38] The demarcation line between oral and pharyngeal tongue lies anterior to the circumvallate papillae; the oral tongue is of ectodermal origin, whereas the pharyngeal tongue is of endodermal origin.[34] The lingual serous glands, thus, are of the same origin as the pancreas.[40] There is also marked similarity in structure between the lingual serous glands and exocrine pancreas; both contain numerous dense, membrane bounded secretory granules.[14,40] Since the secretion of the lingual serous glands is only slightly mucous, it has been proposed that it "serves to wash out the taste buds".[4] Our studies show that these glands secrete a potent lipase which is active in the intragastric digestion of dietary fat. Lingual serous gland lipase activity can be detected 2 days before birth. Whether there is any correlation

TABLE 20–IV

HYDROLYSIS OF CORN OIL-TRIGLYCERIDE IN THE STOMACH OF ADULT RATS

					Lipid composition				
Specimen	*Duration of feeding*	*pH*	*Total fatty acids*		*Tri-glyceride*	*Di-glyceride*	*Mono-glyceride*	*FFA*	*Amount of triglyceride hydrolyzed*
	min		*µEq*	*µEq/g dry weight**	*% of glyceride-glycerol*			*% of total fatty acids*	*µmoles*
Test meal	—	7.0	—	285	90.3	8.2	1.6	0.6	—
Gastric contents									
Rat no. 1	10	4.7	410	316	72.6	23.7	3.8	16.3	24
Rat no. 2	10	4.3	563	268	69.0	27.0	4.4	17.2	40
Rat no. 3	20	4.5	557	396	67.1	29.5	3.4	20.2	43
Rat no. 4	20	5.0	1,700	315	56.4	35.6	8.0	25.8	192

Rats were fed for 2 days a purified diet containing 8% corn oil, 22% vitamin-free casein, 63% corn starch and adequate amounts of vitamins and minerals. They were then fasted overnight and fed the diet *ad libitum* for 10 or 20 min. Gastric contents were then removed for analysis.

* The dry weight of the test meal was equal to 91% of the wet weight, and the dry weight of the gastric contents ranged from 44 to 60% of the wet weight.

(Hamosh and Scow, ref. 17)

between the appearance of taste buds, which are first seen at approximately 20 days of fetal life,[24] and the appearance of the enzyme at the same age is only a matter of speculation. It is of interest that the soft palate also has both lipolytic activity and taste buds.[25]

Evidence of intragastric lipolysis has also been seen in other species. In the dog, 35% of the fat in the stomach was present as FFA 4 hrs after the ingestion of a fat-rich meal. Similar results were obtained in dogs deprived of pancreatic lipase.[11] Marked hydrolysis of triglycerides also occurs in the stomach of calves. The saliva of calves contains a lipolytic activity, called pregastric esterase, which originates in glandular tissue from the back of the tongue, the glosso-epiglottic area of the pharynx and the pharyngeal end of the esophagus.[26-28] In man, Borgstrom and coworkers[6] found that 10–20% of the fat is found as FFA in the stomach 20 minutes after the ingestion of a meal containing 6% corn oil. In addition, diglycerides and FFA were formed when long chain triglyceride was incubated with gastric juice free of duodenal contents from fasting human subjects.[1]

Since the reaction products of lingual lipase are FFA and partial glycerides (70–85% diglyceride and 10–30% monoglyceride), the presence of 10–20% FFA in the gastric contents indicates that 30–60% of the triglyceride has been partially hydrolyzed.

It is generally accepted that the enzymatic digestion of triglyceride starts in the duodenum, and that pancreatic lipase is the key enzyme in the hydrolysis of triglyceride.[21] The only change which is supposed to take place in the stomach is emulsification of triglyceride by the "churning, kneading and squirting movements" of the stomach wall.[20,21,36] Our studies show, however, that dietary triglycerides are rapidly hydrolyzed to partial glycerides and FFA in the stomach. We propose that partial hydrolysis in the stomach is the first step in the digestion of dietary triglyceride and that this reaction is catalyzed by lingual lipase.

SUMMARY

A very high lipolytic activity was found in the serous glands of the tongue in adult and suckling rats. The enzyme hydrolyzes triglyceride to mostly diglyceride and FFA at a pH of 4.5–5.5. The activity is present in the tongue 2 days before birth and increases markedly after birth. The anterior oral pharyngeal wall, lateral oral pharyngeal wall, and soft palate also contained lipolytic activity, but at a much lower level. The lipolytic activity was found in saliva collected through an esophageal cannula and in stomach contents of rats fed a fat-rich meal, as well as in the stomach contents of suckling rats. There was very little activity in the stomach, however, when saliva was excluded. The lipolytic activity was not present in gastric mucosa or in the large salivary glands. The findings suggest that the serous glands of the tongue secrete a potent lipase which acts in the stomach to hydrolyze dietary triglyceride to partial glycerides and free fatty acids. We propose that this reaction is the first step in the digestion of dietary lipid.

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DISCUSSION

Dr. Bosma: A comment about the location of the vallate papillae and von Ebner's glands in the human. In the usual positional arrangement of the infant's mouth, this portion of the tongue approximates the junction of the bony and the muscular palate. During suckle feeding, bolus accumulates in this location, prior to swallow. Accordingly, this is a site of negative pressure during suck. And of positive pressure during the initiation of swallow. I would suggest that these mechanical phenomena may contribute to emptying the secretions of von Ebner's glands from the crevice about the vallate papillae. Thus, the actions of suckle feeding may mechanically facilitate secretion as well as provide a pool in which this secretion is mixed with the bolus.

In postnatal human development, the pharyngeal portion of the tongue is displaced posteriorward and downward in relation to the palate, so that the vallate papillae approximate the muscular palate, in the usual spatial arrangement of the pharynx as it functions as an airway.

Chapter 21

SPONTANEOUS CRY IN THE NEWBORN INFANT; SOUNDS AND FACIAL GESTURES*

Rachel E. Stark and Susan N. Nathanson

BACKGROUND

The aim of the present study is to document the temporal organization of spontaneous crying in the newborn infant (4–19 days) and to describe the auditory and spectrographic features and the facial gestures which are found in different parts of the overall temporal pattern of this crying. Our first task was a methodological one, namely, to find out 1) ways of describing temporal organization in spontaneous cry, 2) what feature judgments it is appropriate to make with respect to individual cry bursts or segments, and 3) in what ways, if any, the facial gestures and postures of the infant relate to these aspects of spontaneous cry.

The ultimate aim of this work is to find out whether changes take place in the temporal organization or segmental features of spontaneous crying as the infant matures; and to what extent these patterns of crying may influence the patterning of non-cry sounds produced in later infancy. We have defined both cry and non-cry in terms of the facial expression of the infant and of the context in which he is vocalizing.[8] Lenneberg[4] has suggested that, although crying undergoes modification during childhood, its development is divorced from that of non-cry sounds 'which eventually merge into the acoustic productions of speech.' Wolff,[11] on the other hand, feels that there is a functional relation between cry and non-cry, and that new non-cry patterns often appear for the first time as the infant produces fussing sounds just before beginning to cry. It is our belief that there is a relationship between cry and non-cry. It may be, however, that the temporal patterning of cry rather than its segmental features, i.e., the features such as diminution of amplitude or pitch break which are applied to individual cry bursts or segments, are reflected in later non-cry vocalization.

Wolff[11] examined the temporal patterning of selected portions of spontaneous crying in relation to the natural breath unit. Prechtl et al.[7] studied the duration of cry bursts and of the intervals between them in regular portions of both pain cry and spontaneous cry, but without reference to respiration. Truby and Lind[9] described different stages, e.g., arousal, attack, cruising, etc., occurring in a typical order in the pain cry

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episode. Our own initial observations suggested that the spontaneous crying of neonates immediately prior to feeding also has typical features of temporal patterning although these are not as clear-cut as in the case of pain cry.

In describing this temporal patterning of spontaneous cry we felt that 1) our description should take into account all of the crying in a given episode, not merely selected parts of it and 2) that in our description both the sounds of crying and the timing of respiration should be taken into account. This led us to the solution of classifying each cry burst on the basis of its duration, intensity, and relationship to a respiratory unit, and then examining ways in which different classes of cry burst grouped themselves over time within a cry episode. The classification also provided a basis for studying different patterns of auditory and spectrographic features found within the cry burst and different combinations of gestures occurring during production of the cry burst.

SUBJECTS AND PROCEDURES

Spontaneous crying was recorded from 8 full-term infants, 5 female and 3 male, whose ages ranged from 4 to 19 days. All of these infants were rated as normal neurologically when examined in the newborn nursery. Their Apgar scores were at least 7 at birth and at least 9 within 3 minutes of birth. Birth weights ranged from 2500 to 3900 grams. Four of the infants were recorded in a quiet room adjoining the nursery. Since we wished to include subjects of more than 3 to 4 days in age, four more infants were recorded after discharge from hospital. In every case recordings were made by means of a Tandberg Type 12 tape recorder and Bruel and Kjaer 1″ microphone, Type 4131. Observation of the infant began 3 to 3½ hours after the beginning of the last feeding and as the infant wakened from sleep and became restless. The infants were lying supine on a flat surface and their outer coverings were removed. The microphone was held 9″ from the mouth when the head was in midline, but since the infant's head was not restrained this distance was not constant but varied by about ± 1″. All of the infants began to cry within 30 to 40 minutes of the beginning of the observation period. A total of 4 to 5 minutes of crying was recorded from each infant. Some infants frequently stopped crying and then started again; others stopped much less often, so that their crying was made up of two or three longer bouts of crying. The range of maximum duration of the bouts of sustained crying, across infants, was 40 seconds to just over 4 minutes. The differences may have reflected the amount of time since the last feed, the amount consumed at that feed, or the infant's success in getting the fist or a portion of blanket into the mouth and sucking on it during the recording session. On the other hand, the work of Aldrich[1] and of Prechtl et al.[7] suggests that there are individual differences of this kind which persist throughout the newborn period. Since we wished to arrive at a way of describing the organization of cry which would cover all cases we did not attempt to control for the variables which might have introduced these differences.

DATA ANALYSIS

Temporal Organization

No portion of the cry episode was omitted from data analysis. In the first stage of analysis, measurements were made of duration and relative amplitude First, however, the units to be measured were defined in the following ways:

1. cry cycle: crying which is bounded at its beginning and end by a silent period, i.e., one in which there are more than two breaths in which no vocalization is produced.
2. breath unit: a unit bounded by the beginning of one inspiration and the beginning of the next.
3. cry unit: a unit bounded by the beginning of an expiratory cry vocalization within a breath unit and the termination of the expiratory vocalization. This cry unit might be divided into sub-units or segments by periods of silence. (See Figure 3).
4. cry segment: sub-portions of the cry unit which are separated from other sub-portions by at least 50 msec. of silence.

Sound level recordings were made for each cry episode in its entirety by means of a Bruel and Kjaer Sound Level Recorder, Type 2305, with the paper speed set to provide a time scale of 30mm=1 second. In addition, sound spectrograms of the entire episode were made by means of a Voiceprint Spectrograph, Series 700, and pasted together to form a continuous record. Each intake of breath was marked on the sound level recording by two listeners independently. Listener agreement of 97% was reached on this task. The beginning of each inspiration was then marked on the spectrographic record. This was done because, while it was more rapid and efficient to make these judgments initially with reference to the sound level recording trace, the spectrographic record provided a greater time scale and an increased number of visual cues signalling the onset of inspiration. This made more accurate measures of duration of the breath unit possible. The duration of each breath unit and of each cry unit was measured to the nearest 10 msec, also the duration of each cry segment and of the silences between them. The measurements were made by two investigators and where agreement of ±10 msec was not reached, the unit was remeasured.

Our attempts to find a basis for classifying the data derived initially from our impressions of the sequence of cry behavior in the spontaneous crying of a number of infants. Typically, it seemed to us, the infant struggled to initiate crying, producing long stressful cries or explosive cough-like sounds in his efforts to do so. These effortful cries were sometimes preceded by low-intensity grunt-like sounds. At the beginning of the entire episode, the infant might not succeed in initiating cry and in this case the effortful cries subsided in further low-intensity sounds and/or silence. Once the infant became more fully aroused, however, crying was sustained. In this case, the initial effortful cries were succeeded by a period of regular, rhythmic crying. This frequently ended in a series of low-intensity sounds as the infant paused to suck on his fist or some foreign object with which his mouth made contact, or as he searched for fist or object after it had brushed his face. He might fall asleep again briefly at this point. However, shortly after losing contact with the fist or object, he would begin to cry again. As the cry episode progressed, initiation of crying appeared to become more efficient and less stressful, provided that the previous cry cycle had been sustained for a sufficient length of time.

When we came to examine the visual records of the crying more carefully, we realized that this was too simplified a view of what was happening. We could, however, still recognize cries which were low in intensity, very long, or regular and began to look in one infant's record for quantitative criteria which were related to these perceptions. Subsequently, we applied the quantitative criteria in classifying the cries from the remaining infants as shown below.

Low intensity cries. It was found that the cry units reliably judged to be less loud than sustained crying were, in fact, at least 10 dB lower in peak intensity than the peak intensity for the infant's entire cry episode. We therefore utilized this criterion for classifying cries as low-intensity cries. Examples are shown in Figures 21–1 and 21–2.

Regular cries. Regular crying was perceived as a portion of the record where inspiration followed expiration, and expiration followed inspiration without marked pause; also the cry units in regular crying were not broken up by silence

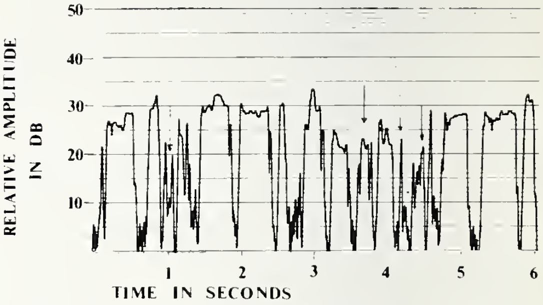


Figure 21–1. Sound level tracing (made at 10 mm per second) of a portion of a cry cycle from infant M.P.V. The arrows point to low intensity cry units.

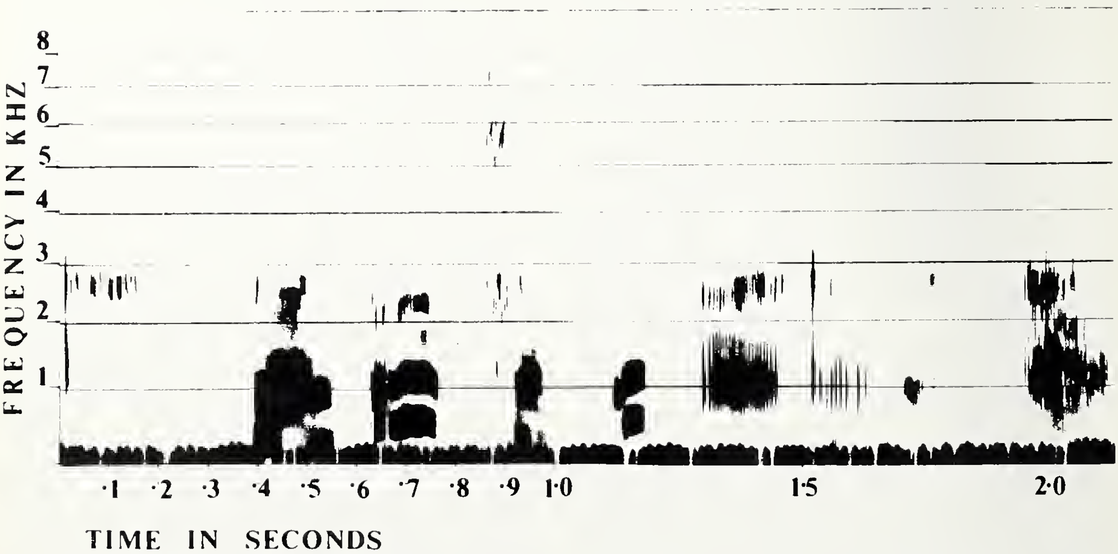


Figure 21–2. Wideband spectrographic record of a sequence of low intensity cry units occurring in mid-cycle in the recording of infant A.G. Voicing change is present in the third cry segment (beginning at .9 seconds). This segment is followed (1) by a high-pitched voiced inspiratory sound and (2) by two segments which are characterized by vocal cry.

or a marked decrease in loudness in mid-cry.

It was accordingly decided that in regular crying:

1. the inspiration at the beginning of the breath unit must not be separated from the beginning of the cry unit by more than 30% of the total breath unit duration.
2. the inspiration following the cry unit must occur within 100 msec. of the end of the cry unit.
3. there must be no silence in mid-unit and no dip in amplitude with a depth of 6 dB and a slope of 3 dB per mm on both sides of the dip.

Examples of regular cries are shown in Figures 21–3 and 21–4.

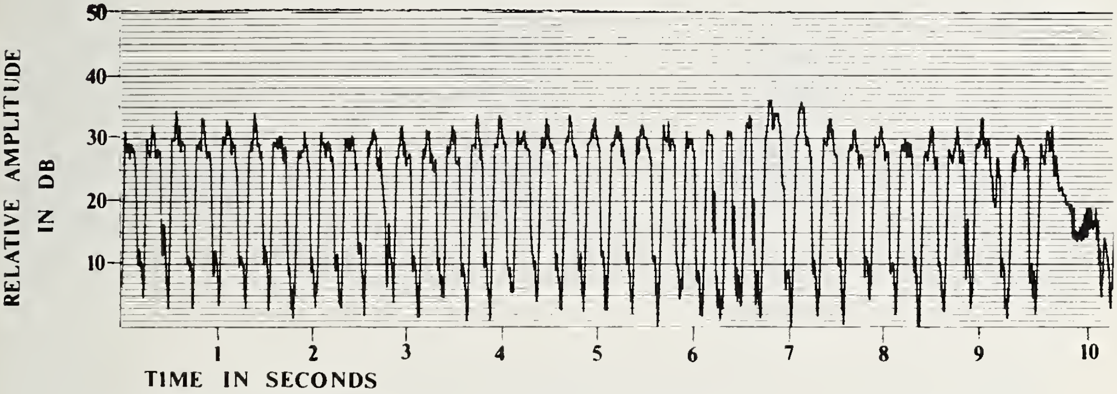


Figure 21–3. Sound level tracing (made at 10 mm per second) of a portion of a cry cycle from infant R.W. This shows a sequence of regular cries followed at about 6 seconds by 3 'other' cries, each of which is terminated by forceful expulsion of air. A second sequence of regular cries (with one 'other' at 9 seconds) is followed by a long non-low cry at 9.5 seconds.

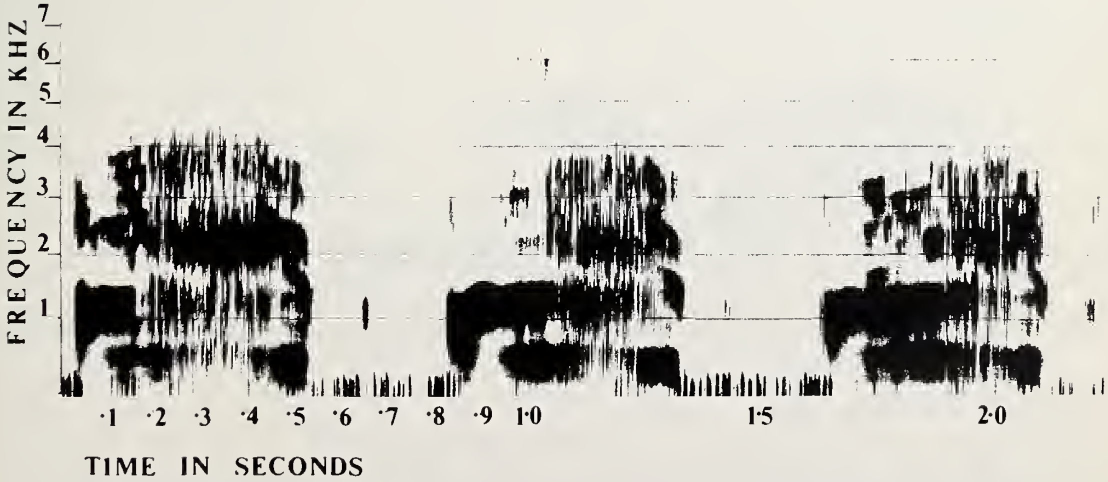


Figure 21-4. Wideband spectrographic record of three regular cries from infant R.W. Each cry unit shows a rapid rise in pitch at the beginning, a rapid fall in pitch at the end, and turbulence in mid-cry.

Long, non-low cries. These were cries where the breath unit duration was greater than for the longest of the cries classed as regular, and which had not already been classed as low-intensity cries. We have referred to them in the text as long cries for the sake of brevity, but as long, non-low cries in the Figures and Tables. Examples are shown in Figures 21–5 and 21–6.

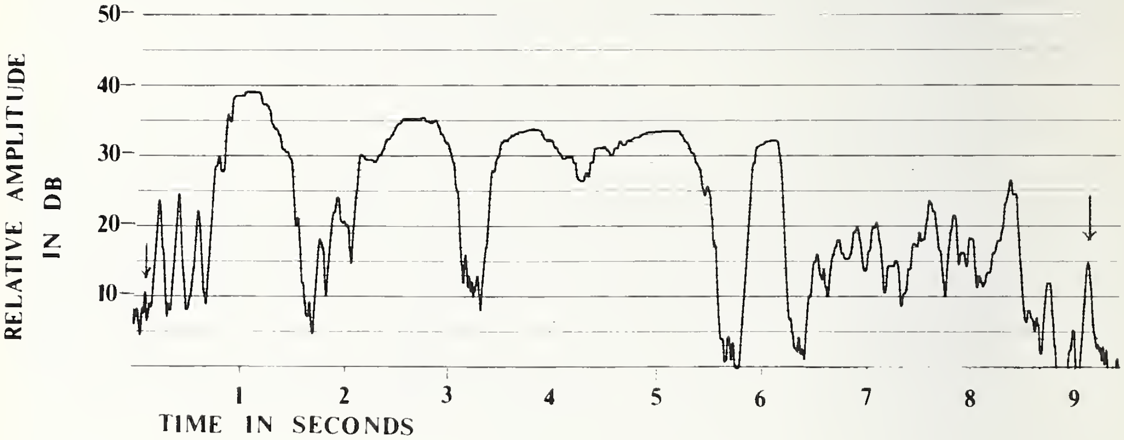


Figure 21–5. Sound level tracing (made at 30 mm per second) of a long non-low cry unit from infant G.D. The inspiration at the beginning and end of the breath unit are marked by arrows. This cry unit, which initiates a cry cycle, is broken up by marked dimunition of amplitude (e.g., in the first second) and silence (e.g., at 5.8 seconds and 6.3 seconds).

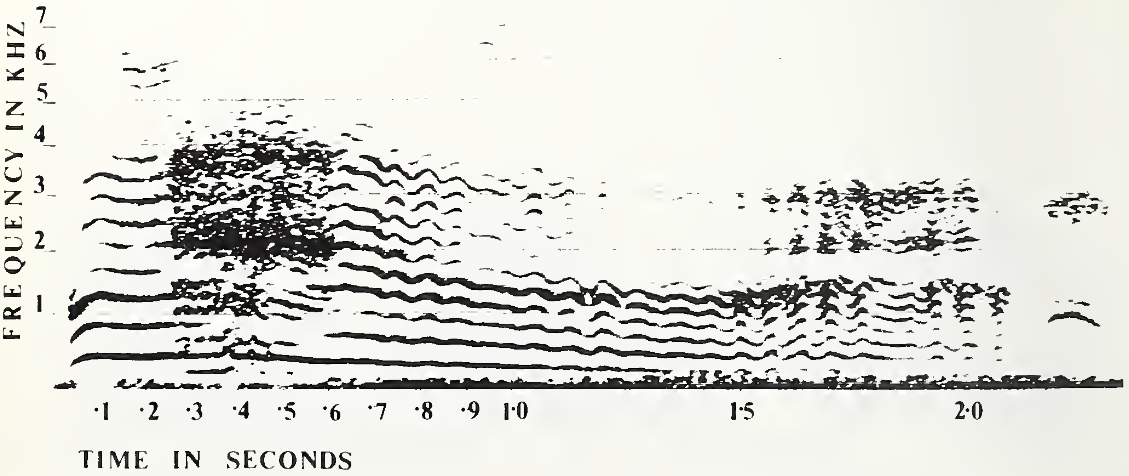


Figure 21–6. Narrowband spectrographic record (45 Hz bandwidth) of a long non-low cry unit from infant R.W. This cry unit occurred in mid-cycle. In its first portion it resembles a regular cry with a rapid rise in pitch at onset and turbulence and subharmonic break from .25 to .6 seconds; this is followed by a long portion characterized by vibrato. A voiced inspiration follows the cry unit.

Other cries. The remainder of the cries were all, by definition, of lesser duration than the long cries, and they had not been classed as regular or as low in intensity. They were simply classed as "Other". Examples are shown in Figures 21–7 and 21–8.

In addition, breath units in mid-cycle

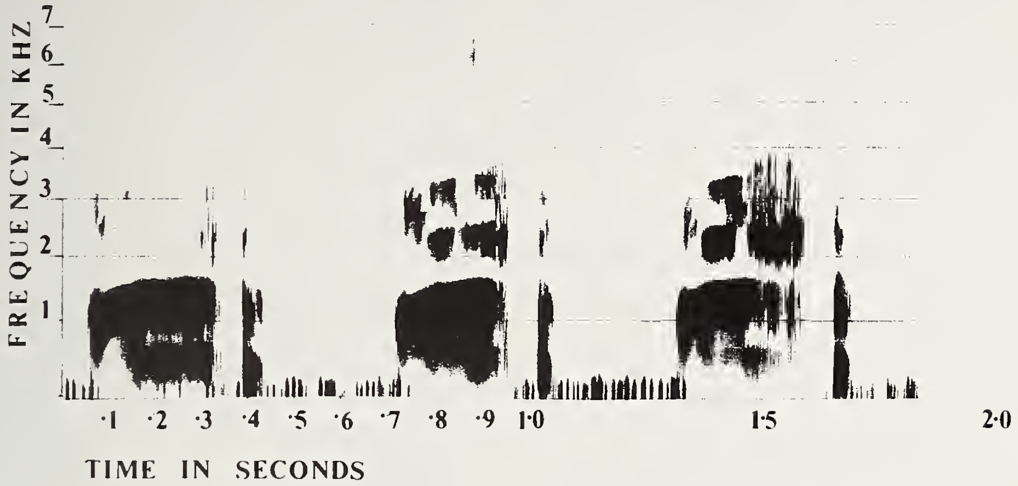


Figure 21–7. Wideband spectrogram of 3 'other' cry units from infant R.W. These cries occurred in mid-cycle, but are similar to 'other' cries which initiate a cry cycle. They resemble a regular cry in their initial to mid portion but terminate with forceful expulsion of air.

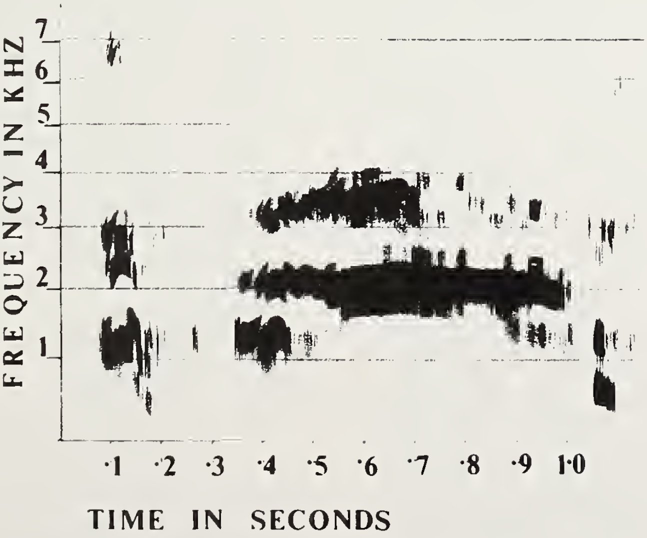


Figure 21–8. Wideband spectrogram of an 'other' cry unit from the record of infant M.W. This cry has two segments separated by silence. Rapid pitch change is present in both segments. The breath unit in which the cry occurs terminates with a voiced inspiration.

in which no vocalization occurred were classified separately and referred to as empty breaths.

The means and standard deviations for the breath unit and cry unit duration of each of these cry types and for all 8 subjects are shown in Tables 21–1 through 21–8. Considerable variation may be observed from infant to infant as well as some commonalities. These are summarized as follows:

1. Breath unit duration. For all infants, mean breath unit duration is highest for long cries; the range and standard deviation are also greatest for this category except in infant A.G. Regular and 'other' cries are next highest and low intensity cries have the least duration, except for infant V.W., whose mean duration for low cries is longer than that for regular cries.
2. Cry unit duration. Cry units are identified in terms of the breath unit as stated above. Mean cry unit duration for the various categories of cry unit is not dependent on category definition in the same way as mean breath unit duration. Yet the data for cry unit duration show more consistent relationships across cry types than the breath unit duration data. For every infant long cries are longest, low intensity cries are shortest and the regular and 'other' cries fall between these extremes. As a corollary of this ordering, it may be observed that low cries occupy a smaller percentage of the breath unit than any other category, except in infant V.W.

In addition, we have noted consistent variations of unit duration within categories in some infants which are not shown in these Tables. For example, in those infants who initiated crying with long cries, these cries tended to be longer at the beginning of the cycle than in mid-cycle. Also, in 6 out of the 8 infants regular cry units tended to become shorter toward the end of the cry episode than they were at the beginning.

Data on the percentage of cry units placed in each category are summarized across infants in Table 21–9. Here again there is considerable variability across infants although all of the 4 cry types we have specified were represented in every infant's record. It is possible that some of the variation in distribution of cry types is related to degree of arousal since it is our observation that the crying of an infant who is fully aroused is more regular and rhythmic than the crying of one who is not. It is also possible that some of the variation in distribution of cry types is associated with the degree of maturity of the infant. For example, B.H., the youngest infant in our sample, produced long series of brief low-intensity cries (mean duration 213 msec.). Aldrich[1] commented that this was typical of some of the infants he observed in the newborn nursery. Infant M.P.V., who appeared to have much greater variety and control in her crying also produces a high percentage of low cries; but these do not occur in long series and they are of greater duration (381 msec.) than for infant B.H. In the two infants with the highest percentage of 'other' cries, i.e., female infants M.P.V. and A.G., the percentage of long cries is smallest. These 2 infants initiated the cry cycle with cry units in the 'other' category; their initiating 'other' cries were shorter than the 'other' cries of the remaining infants and were explosive and cough-like in nature, i.e., they were accompanied by the forceful expulsion of air described in more detail below. Most of the remaining infants initiated crying with long cries. Since it is our observation that within a few months most, if not all, infants begin to produce short explosive sounds accompanied by forceful expulsion of air in some parts of their crying, we have speculated that the use of this mechanism in neonates may be evidence of maturity.

We next examined the record of each infant to discover the ways in which these cry types succeeded one another within a cry cycle. These data are summarized for 3 different infants who are representative of the sample in Figure 21–9. The cry categories which comprise each one-third portion of the episode are listed on the left. Successive cry units which were classified alike are grouped together and their number expressed as a percentage of the total episode. Thus, each portion of the record is read from left to right and the 3 portions from the top downward. A diagonal slash mark indicates the end of a cry cycle. These records indicate that the infant does not continue to cry regularly after an initial struggle period but instead shows a great deal of fluctuation between different cry types in his sustained crying. For example, regular and 'other' cries frequently alternate in mid-cycle; where the 'other' cries are non-explosive, crying of this kind is more varied in character than in the case of a series of regular cries only, but the crying may still be perceived as relatively rhythmic. On the other hand, long, or explosive 'other' cries in sustained crying give the impression of a change in rhythm suggesting a relative increase in level of distress.

For infant V.W. (see Figure 21–9a) a number of short cry cycles are succeeded by two longer cry cycles. It will be observed that at the beginning of each cycle but one, the sixth, there is a series of long cries or long cries preceded by low-intensity cries. In fact, the brief cycles may consist only of long cries or of long and low-intensity cries in some combination with one another. Once this infant is more fully aroused the cycles lengthen and regular and 'other' cries appear in mid-cycle.

In the records of M.P.V. it will be observed (see Figure 21–9b) that there are three longer cycles, each one beginning with 'other' cries or 'other' cries preceded by low-intensity cries. The 'other' cries of this infant, as already indicated, were frequently explosive in character and accompanied by a forceful expulsion of air. The 'other' cries in mid-cycle frequently followed low cries and appeared to signal a recurring increase in distress, just as the long cries appeared to do in the case of infant V.W.

In the first cycle recorded from infant B.H. (see Figure 21–9c), the long series of brief low-intensity cries resembled grunts produced in non-cry vocalization. In common with the 2 infants shown in Figure 21–9a and 21–9b, B.H. produces many more regular cries in the later cycles than in the earlier cycles.

In summary, we may say the following with respect to the temporal organization of spontaneous cry.

1. This type of crying is initiated by long stressful cries, or by 'other' cries which are frequently characterized by forceful expulsion of air. These initiating cries may be preceded by low intensity sounds.
2. Regular cries do not occur at the beginning of a cry cycle; they frequently alternate with 'other' cries in mid-cycle and may terminate a cycle; in this case, crying is perceived as ceasing abruptly.
3. Low intensity cries may occur at the beginning of a cycle, or in mid-cycle; in mid-cycle they appear to

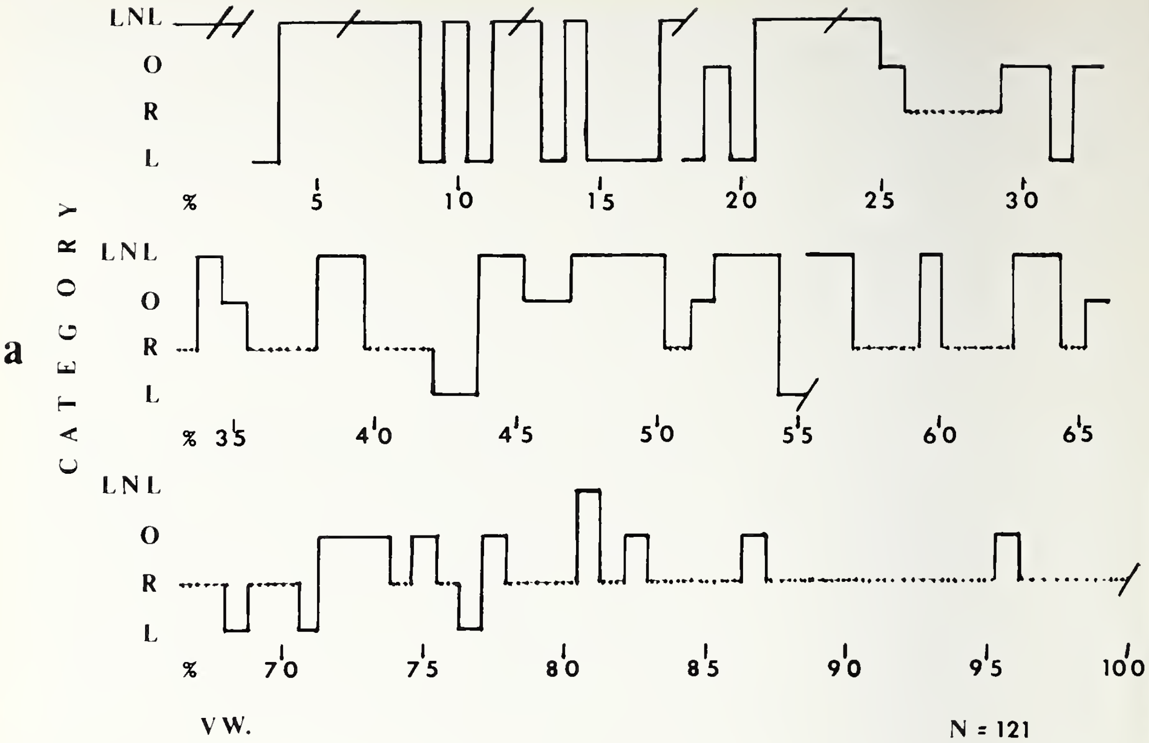


Figure 21–9. Temporal organization of the spontaneous crying of 3 different infants (a) V.W., (b) B.H. and (c) M.P.V. The number of contiguous cry units falling into the same category has been expressed as a percentage of the entire cry episode. A diagonal slash mark indicates the end of a single cry cycle; a horizontal line indicates a group of cry units which were classed alike in the order in which they occurred, and a vertical line, a change in mid-cycle from one category of cry to another. A broken horizontal line indicates a group of regular cries, which may thus be visualized more readily. The number of units in the entire episode is shown in the lower right hand corner.

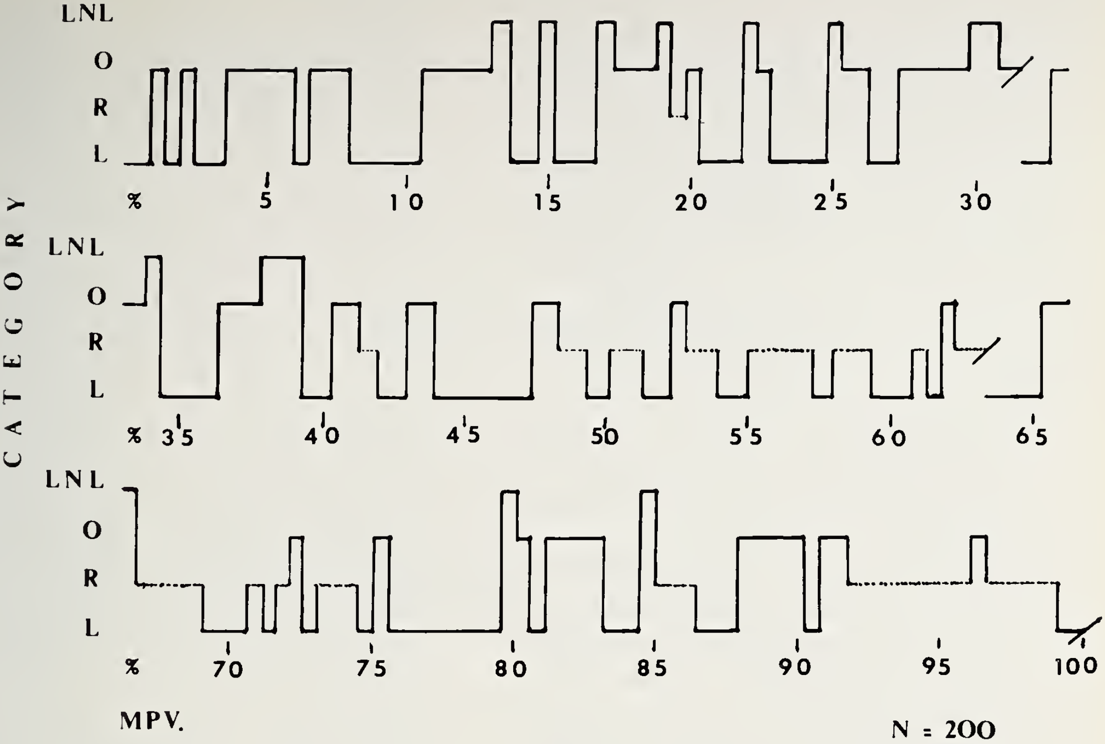
signal a decrease in level of distress; they may terminate cycles of any length, that is, whether or not regular crying has been established.

4. Long, non-low cries may occur at the beginning of a cycle or in mid-cycle; in mid-cycle they appear to signal an increase in level of distress; they do not terminate a cycle unless it is very brief, i.e., in the case where regular crying has not yet been established.
5. 'Other' cries may occur at any point in a cry cycle; the longer a crying episode continues the more likely it is that regular and 'other' cries will both occur with high frequency, often in alternation with one another.

Auditory and Spectrographic Features

In the second stage of data analysis, judgments about certain auditory features of cry were made by two listeners who used high quality headphones.

b


CATEGORY
LNL
O
R
L
%
5
10
15
20
25
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45
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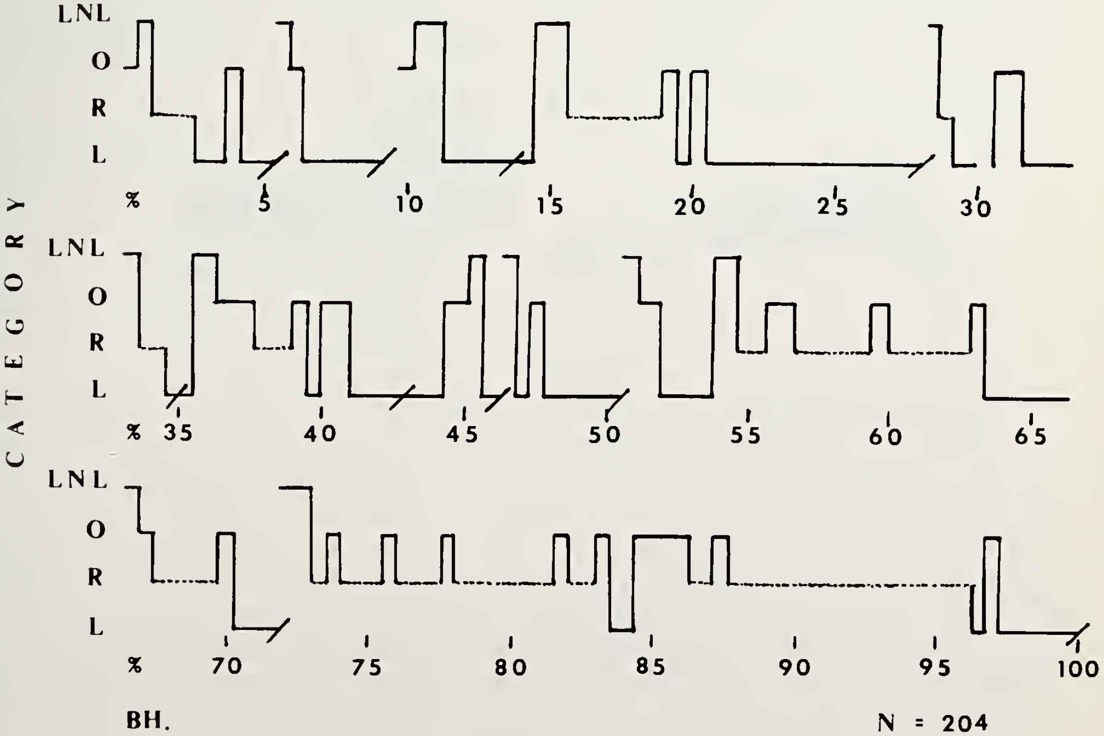
55
60
65
70
75
80
85
90
95
100
MPV.
N = 200

c


CATEGORY
LNL
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65
70
75
80
85
90
95
100
BH.
N = 204

These listeners at the same time examined the spectrographic record and sound level recordings of the cry unit to be judged. Judgments were made about the presence or absence of voicing, forceful expulsion of air, closure of the vocal tract above the glottis, and a set of voice quality features. Most of the voice quality features we included have been proposed by other investigators.[5,6,10,11] Definitions for the entire set of features which we used are as follows:

1. Vocal fry (VFRY). A low-frequency glottal pulsing which is variable in rate, shown in Figure 21–2.
2. Pitch shift. A sudden change or break in pitch, such that the pre- and post-shift portions of cry are not harmonically related (see Figure 21–10).
3. Turbulence or noise (TURB). Aperiodic sound produced above the level of the glottis (see Figure 21–4). This is referred to by Truby and Lind[9] as dysphonation. In our sample we have sometimes found this to be associated with a doubling of harmonics, a phenomenon which Wasz-Hockert et al.[10] referred to as subharmonic break. We do not know the glottal or supraglottal mechanisms to which this is related (see Figure 21–6).
4. Diminution of amplitude (DIMAMP). A dip in amplitude on the sound level recording such that the event occupies no more than 20–30 msec with a steep rise of at least 6 dB on either side of the dip (see Figure 21–5).
5. Voicing change (VCHG). The alternation of a voiced portion of cry with a voiceless portion. This frequently occurs at the beginning of a cry unit where it sounds /hæ-like, or at the end where it sounds/æh/-like (see Figure 21–2).
6. Forceful expulsion of air (FEXP). This is perceived as being an alternation of voiced and voiceless portions of cry which are accompanied by a forceful expulsion of air. In these cases a direct observation of the infant suggests that air is forced through the glottis as a result of a spasmodic movement of the diaphragm; the folds may continue to vibrate in the air stream as they are blown apart and forcefully brought back together again. The onset of the following voiced portion is very abrupt. This phenomenon is referred to by Wasz-Hockert et al.[10] as 'glottal pulsing' (see Figure 21–7).
7. Rapid pitch change (RPC). The abrupt change of fundamental frequency often coinciding with abrupt onset or cessation of a sound from or to a position of glottal closure (see Figure 21–4 and 21–6).
8. Vibrato (VIBR). A series of rapid pitch changes (see Figure 21–6).
9. Voiced inspiration (VINSP) (see Figure 21–6).
10. Silence (SIL). A silence in mid-cry unit. By definition this would be 50 msec. or less in duration (see Figure 21–8).
11. Closure of the vocal tract above the glottis (CLVT). The forming of a constriction greater than that necessary to produce a glide, by approximation of tongue, palate, velum or pharyngeal wall. We have not encountered a bilabial or tongue-tip closure in the spontaneous crying of neonates (see Figure 21–11).

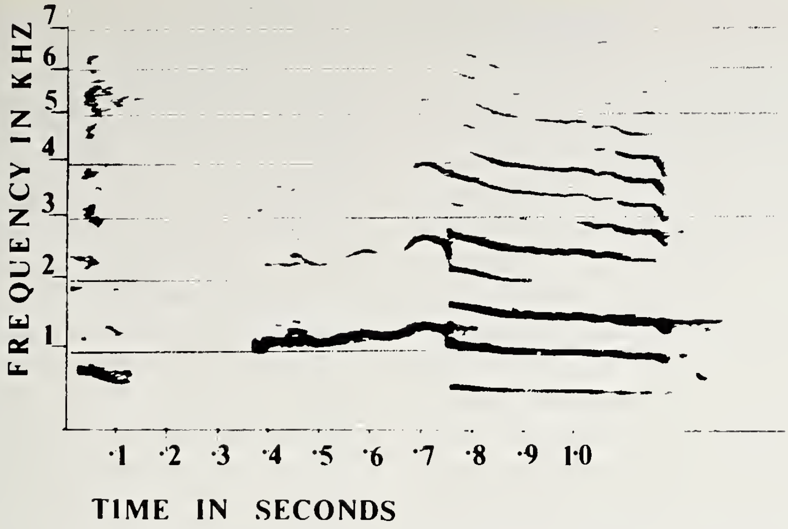

Figure 21–10. Narrowband spectrogram of a regular cry unit from the record of infant B.H., showing a rare example of pitch shift.

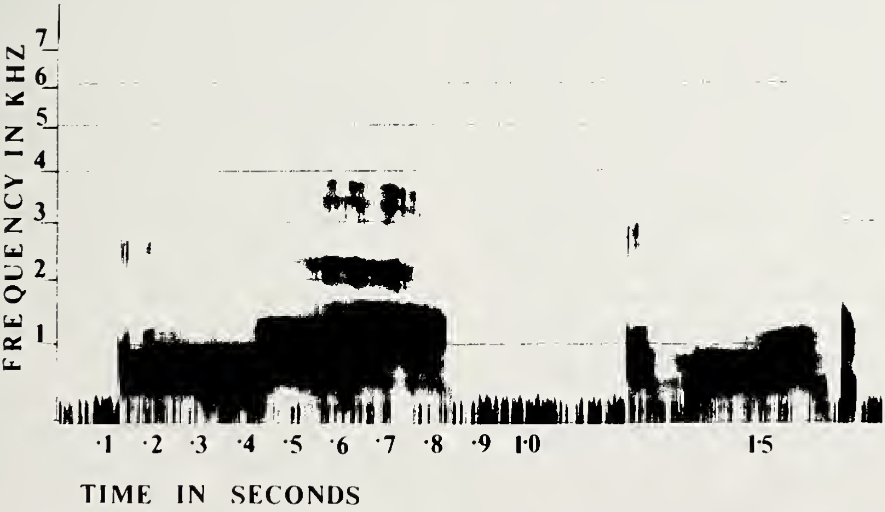

Figure 21–11. Wideband spectrogram of a regular cry unit followed by a low-intensity cry unit in the record of infant R.W. Closure of the vocal tract occurs at the beginning of the regular unit. This closure, which is illustrated further in the cine frames of Figure 12 (a) has a /n/-like quality. It is released at .42 seconds.

The judgments were made so that we might find out if the 4 cry types differed from one another in the feature sets of which they were made up either within individual infants, or across infants. Also the analysis was designed as a first step toward finding out which features of cry are also present in non-cry. The feature description was relatively gross, however, since no attempt was made to keep track of the orders in which features occurred within a cry unit. Inter-judge agreement was found to be 81%.

The frequency of occurrence of the features across cry types is shown for 4 different infants who are representative of the group in Table 21–10. From this Table it would appear that the correlation between cry category and presence of individual features is not very high. It is possible that sets of features occurring in a given temporal relationship to one another do correlate more highly with cry category than single features. This can only be determined by further work. However, even at the present level of analysis, it can be seen that certain features are typical of a single cry category in more than one infant. For example, voiced inspiration is more often found in regular cry units than in any other category of unit in all 4 infants. Similarly vocal fry is more often found in long cry units than in any other category in all 4 infants. Notice also that, for all 4 infants low-intensity cries are characterized by a few features most of them having a low rate of occurrence; regular cries are more stereotyped than cries in other categories, i.e., they are characterized by a small number of high-frequency features; and long and 'other' cries are characterized by a greater variety of relatively high frequency features than regular cries or low intensity cries.

Table 21–10 also gives evidence of differences between infants. For example, forceful expulsion of air is found in the cries of infants A.G. and M.P.V., chiefly as stated above in the 'other' cry category but also in the low intensity category. It is not found in the cries of the other two infants shown. Similarly, closure of the vocal tract occurred in all cry categories in 3 of the infants but in no cry category in the fourth infant, M.W. From a limited amount of longitudinal data on 3 of our subjects, we believe that these differences between infants are reliable and persist at least for the first few weeks of life. On the other hand, some features, such as rapid pitch change, have a relatively high frequency of occurrence across categories in all infants while other features such as pitch shift have a relatively low frequency of occurrence across categories in all infants.

These findings suggest that:

1. the features of spontaneous crying are different from those of pain-elicited cry. For example, pitch shift, which we found to have a low frequency of occurrence in spontaneous cry, has been reported as having a high frequency of occurrence in pain cry. The reverse is true of closure of the vocal tract.
2. individual feature differences together with differences in the proportion of usage of cry categories, unit duration, and cycle duration could contribute to a mother's recognition of her own infant's cry, provided that she heard a long enough sample from his crying.
3. across infants there are certain commonalities in spontaneous crying. For example, regular crying is relatively stereotyped, i.e., it is characterized by relatively few features, one of which is voiced inspiration, while low intensity cries are relatively featureless. Also certain features such as rapid pitch change are found with a high rate of occurrence across infants and categories.

FACIAL AND OTHER GESTURES

One of our aims in making a study of the facial gestures which accompany spontaneous cry was to find out if differences in stress or tension of the infant were reflected in the auditory and spectrographic features of crying. We wished also to find out if closure of the vocal tract was taking place at those points where we perceived closure on listening to the cry, and where we thought we saw evidence of closure in the spectrogram.

A videotape recording was made of the spontaneous crying of one infant (R.W., 14 days) with the aid of an Ampex 7800 Videotape Recorder. This was later converted to 16 mm sound film made at 24 frames per second. The entire film has been examined in some detail with the aid of a Tagarno 16 stop-frame projector.

This infant produced 2 cry cycles. Before she began to cry, she succeeded briefly in touching her mouth with the right hand. Throughout the first cycle her head is turned toward her right. During this cycle she does not become extremely tense. There is considerable movement of the upper and lower extremities, the mouth is only partly open and the tongue at times moves independently of the jaw. It moves in an upward and downward direction and sometimes anteriorly. Some of the movements appear to be like those made preparatory to feeding.

During the second cycle, this infant becomes much more tense. The head is turned toward the midline, and the body appears quite rigid. There is much less movement of the upper and lower extremities and indeed the hands are held in a tense clasped position much of the time. The mouth is held wide open during the entire sequence and the tongue is mostly held in a cupped or concave shape. There is much less movement of the tongue and jaw than during the first cycle.

Two separate cry units have been selected to illustrate differences between the 2 cycles. One of the units is a regular cry unit (θ 25) produced towards the beginning of the first cycle when the infant was not fully aroused. The second is a long non-low unit (θ 211) produced toward the end of the second cycle when the infant was fully aroused. The sequence of frames illustrating facial and other body gestures accompanying these cries are shown in Figure 21–12 and 21–13 respectively.

In the case of the regular cry unit (Figure 21–12), the mouth is partly open just before the onset of the cry, i.e., the seal between the tongue and the palate which is characteristically present during non cry, is broken. However, during the first part of the cry the mouth closes slightly as a result of upward movement of the jaw, the tongue moves upward with the jaw, makes contact with the anterior part of the hard palate and remains in contact with it for almost 400 msec. The closure of the vocal tract is perceived as /n/-like. A wideband spectrogram of the cry is shown in Figure 11. It is not a tense cry—note the lack of turbulence in its mid portion. The nasal closure is reflected in the distribution of energy in the first part of the utterance and the release of the closure can be seen at .4 seconds in the spectrogram. This coincides with frame θ 20. In Frame θ 21 the descent of the jaw and the breaking of the contact of tongue and palate can be seen quite readily. The tongue continues to descend toward a neutral position in the mouth over the next 8 frames and a slight difference in configuration of the lips and the tongue on the right and left side become noticeable. By the end of the regular cry the jaw has returned to a more neutral position and the mouth is more nearly closed. Both upper limbs move during this cry, the right more than the left.

The long cry unit illustrated in Figure 21–13 was of 4 seconds' duration. During its production the head is in the midline and thrust backward to some extent. There is very little movement of the upper extremities. The mouth is held wide open during the entire sequence. A wideband spectrogram of a very similar but shorter cry, (#205), is shown in Figure 21–6. Like the cry shown in Figure 21–6, the present cry unit begins with a portion which resembles a stressful, regular cry, i.e., one in which turbulence is prominent; however, it ends with a long, tense vibrato portion. The sequence of cine frames shown in Figure

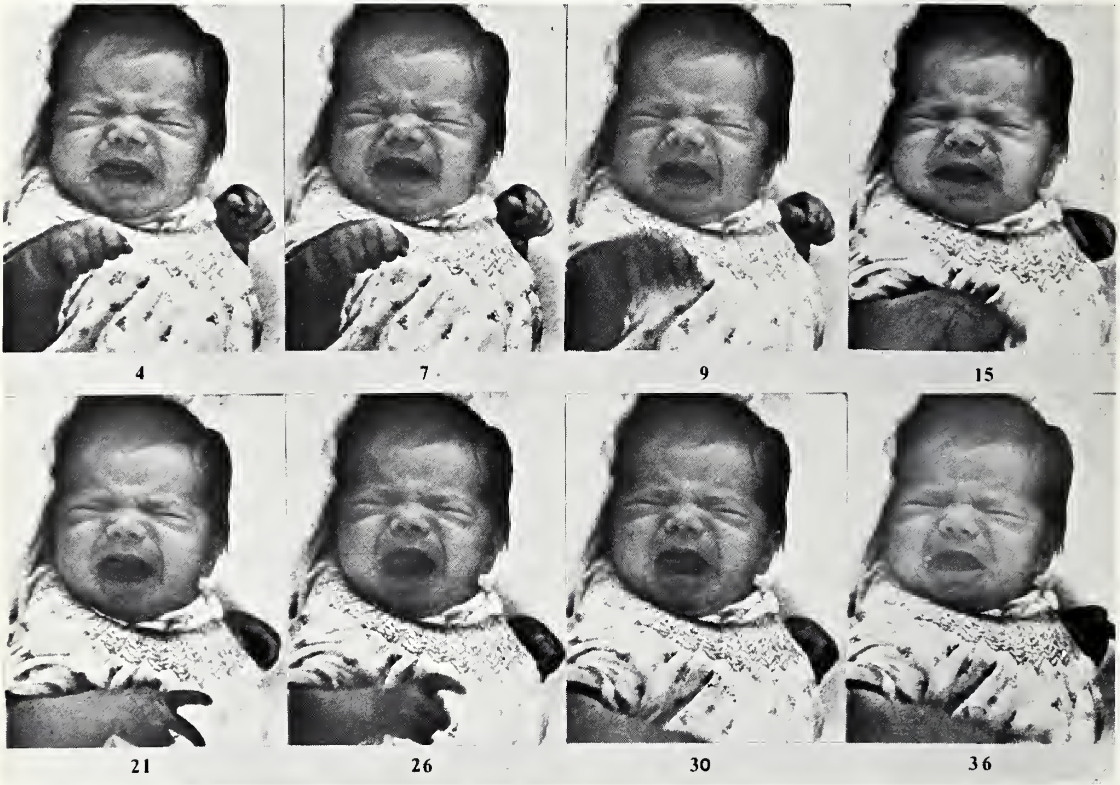

Figure 21–12. Frames taken from a cine photographic sequence depicting a regular cry from the record of infant R.W. Towards the beginning of this sequence (Frame #4) the tongue is in a concave shape in a neutral position in the mouth. In Frame #6 the jaw has assumed a higher position and the mouth is more nearly closed. The tongue continues to move upward with the jaw and in Frame #15 it is out of sight and in contact with the anterior part of the hard palate. This closure is perceived as a /n/-like sound. In Frame #21 the tongue has begun to descend with the jaw so that by Frame #26 the mouth is more widely open. Notice that the tongue appears to be asymmetrical in Frames 26 and 30, that is, the right half appears lower than the left. By Frame #35 the tongue and jaw have returned to a neutral position.

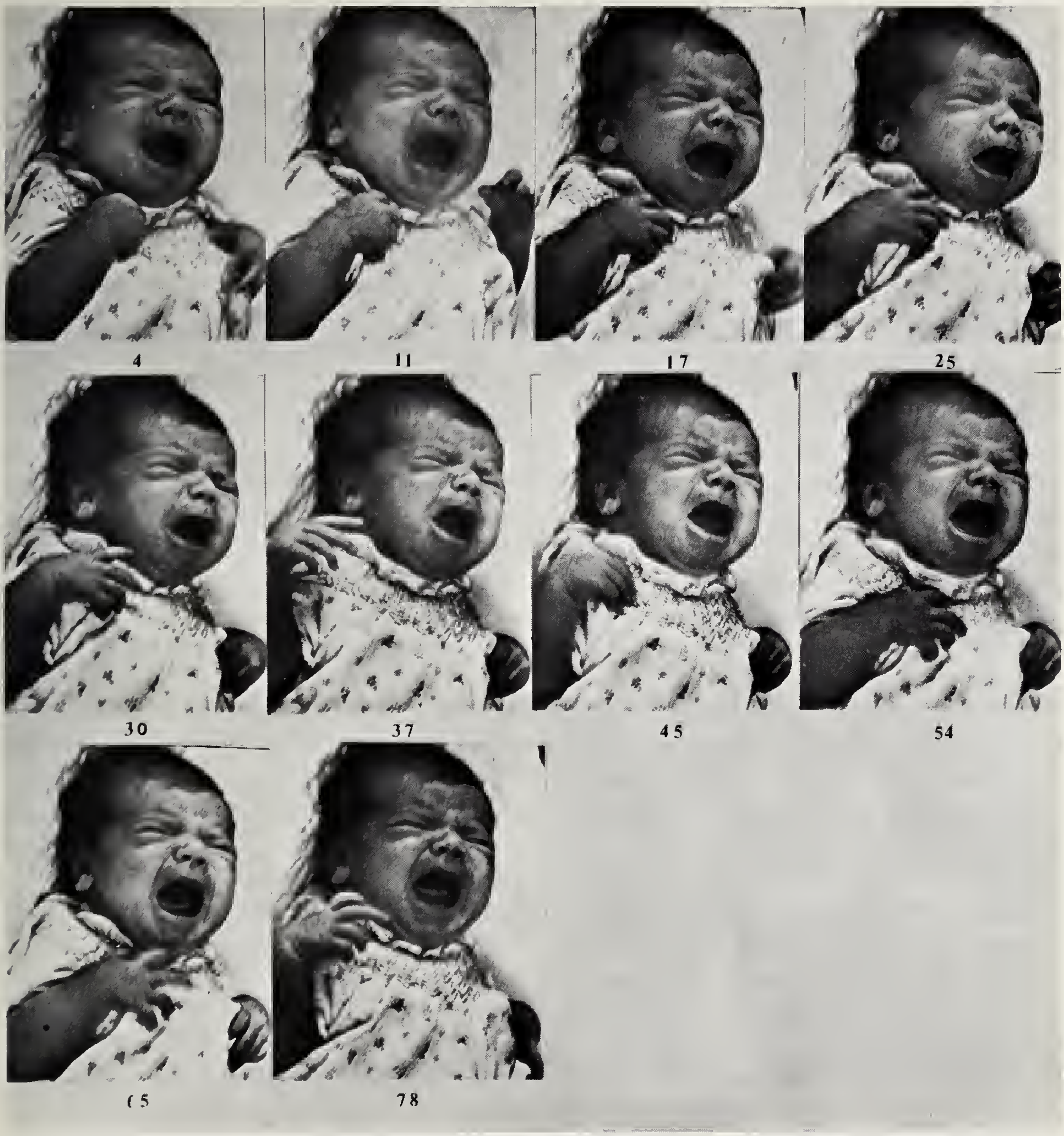

Figure 21–13. Frames taken from a cine photographic sequence depicting the latter part of a long non-low cry from the record of infant R.W. Tongue and jaw position change very slowly in this sequence and the body and limbs are stiff and move little. The mouth is wide open throughout. The tongue flattens in Frame #30 and, during the tense, vibrato portion of the cry, it lowers to a point behind the lower gum ridge. From Frame #54 the tongue rises up again and by the end of this sequence it has resumed the concave shape which it had before this cry began i.e., about 12 frames prior to the sequence shown.

21–13 is of this latter part of the cry. There is no closure of the vocal tract in this cry; instead, during the vibrato portion the tongue flattens and descends to the floor of the mouth. This tongue movement appears to be independent of jaw movement. The tongue gradually returns to a mid position and resumes a concave shape.

It is our feeling that auditory features do correlate quite highly with face, mouth, and body gestures. The closure of the vocal tract shown in Figure 21–11, for example, has correlates in the tongue movement sequence shown in Figure 21–12. Certain voice quality features such as turbulence and pitch break may correlate highly with the bodily tension reflected in the opisthotonic posture of the infant. However, such voice quality features must also correlate with gestures in the region of larynx and pharynx which we cannot visualize.

we cannot visualize.

Bosma, Truby and Lind[2] have observed in the case of pain-elicited cry, that movements of tongue and jaw appear to be out of phase with laryngeal action. We have found the same to be true of spontaneous cry. The downward movements of tongue and jaw during cry do not coincide with, but occur subsequent to the onset of phonation. Similarly, the elevation of tongue and jaw at the end of a cry does not coincide with, but follows, the end of the expiratory phase of the cry.

SUMMARY AND CONCLUSIONS

We have attempted to classify different cry units in terms of amplitude, their relation to the natural breath units in which they occur and the duration of that breath unit. We have used this classification as a means of describing the temporal organization of spontaneous crying and of examining the auditory and spectrographic features of different types of cry unit. We have also begun to look at the facial gestures and head and body postures which typically accompany spontaneous cry, and at the ways in which these gestures relate to the sets of auditory and spectrographic features recorded.

It is our impression that head and body postures are related at least indirectly to certain auditory and spectrographic features of cry. When these postures indicate that the infant is in a more relaxed state certain features, such as closure of the vocal tract, may have a higher probability of occurrence. Conversely, when these postures indicate a tense state other features, such as pitch shift or turbulence, may have a higher probability of occurrence. Other features may relate to the management of respiration. In initiating cries, for example, the infant appears to close the glottis firmly during the expiratory phase and at the same time to exert a positive pressure below the glottis by means of contraction of the body wall and the elastic recoil of the lungs. The infant's efforts, with the features of silence, vocal fry and forceful expulsion of air, appear to be directed toward resolving these antagonistic forces.

The relationship of head and body posture to cry category is less clear. Low intensity cries appear to occur only when the infant is less tense. However, long, regular, and 'other' cries may be produced in a variety of states. If so, it becomes interesting to ask how the features or sets of features found in these cry types vary with state. Also the temporal organization, i.e., the way in which cry categories group themselves within a cycle and their relative frequency of occurrence, may well be related to state.

The effects of state and of the demands of the respiratory system may interact in such a way as to produce individual patterns of cry. Also, these effects may result in some degree of cry differentiation even in the neonate. Certainly, as a result of the work accomplished thus far, we have been able to specify individual characteristics of a kind which mothers might be able to use in recognizing their own infants. We have also found that, even in the first week of life, spontaneous crying is quite different from the pain-elicited cry of the neonate described by Truby and Lind[9] and others. It differs from pain-elicited cry in its temporal organization and in the set of features of which it is made up; that is, some features are common to both types of cry while others are not. In the past, this differentiation has frequently been reported as appearing at about 4 weeks of age.[3] It is possible that cry differentiation increases with age over the first 3 weeks of life. We suspect also that parents may be learning to recognize different types of cry during those first weeks.

The descriptive approach which we have developed may be used to find out 1) whether cry sounds show developmental changes over the first weeks of life and 2) whether any aspects of spontaneous crying are to be found in non-cry vocalizations produced after about 8 weeks of age. We have already begun to ask such questions in examining both longitudinal and cross-sectional data obtained from young infants.

Finally, although we are a long way from understanding the physiological and neurophysiological mechanisms of cry production, i.e., how some of the features we hear and see are being produced, or how the infant's basic functions are affected by crying, we have learned something from careful description of the behavior. When techniques do become available which can be used without hazard to the infant for measuring air flow or muscle activity, for example, or for obtaining information from fiber optics or cineradiography, we shall probably make use of such descriptive techniques in interpreting the new data. In this way description becomes a useful preliminary to studies of the mechanisms of cry production, studies which will eventually further our understanding of the development of speech.

TABLE 21–1
SUMMARY OF UNIT DURATION MEASURES FOR INFANT V.W., AGE 19 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	16	3800– 610	1658.13	863.14	
Regular	49	2500– 830	1393.06	428.93	
Long non-low	35	7560–2550	3664.86	1125.89	
Other	19	2500–1010	1783.16	479.50	
Empty breaths	4	190– 900	402.50	341.30	
			Cry Units		
Low	16	2000– 70	655.00	474.96	40
Regular	49	2110– 480	971.22	392.99	70
Long non-low	35	6780– 580	2675.14	1182.96	73
Other	19	2080– 580	1314.74	437.80	74

TABLE 21–2
SUMMARY OF UNIT DURATION MEASURES FOR INFANT M.P.V., AGE 7 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	82	1370– 320	816.95	243.81	
Regular	45	1880– 450	1302.22	306.53	
Long non-low	15	3400–1920	2415.33	435.66	
Other	58	1640– 470	884.31	277.82	
Empty breaths	2	560– 360	460.00	141.42	
			Cry Units		
Low	82	1110– 20	380.98	265.12	47
Regular	45	1540– 240	995.11	281.87	76
Long non-low	15	2480– 660	1990.67	572.11	82
Other	58	1270– 120	477.59	263.72	54

TABLE 21–3
SUMMARY OF UNIT DURATION MEASURES FOR INFANT G.D., AGE 3 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	54	4690– 390	967.77	690.79	
Regular	50	2350– 780	1487.60	351.08	
Long non-low	34	9100–2370	4037.94	1456.30	
Other	15	2340– 770	1653.33	536.39	
Empty breaths	7	750– 240	427.14	210.21	
			Cry Units		
Low	54	4580– 50	525.74	682.67	54
Regular	50	1850– 510	1051.00	282.20	71
Long non-low	34	8820–1310	3251.10	1545.90	84
Other	15	1540– 510	1119.16	377.40	71

TABLE 21–4
SUMMARY OF UNIT DURATION MEASURES FOR INFANT J.G., AGE 7 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	27	3650– 200	937.78	673.00	
Regular	49	3950–1390	2677.55	629.62	
Long non-low	15	12680–4050	5814.67	2171.33	
Other	40	3930–1090	2690.00	853.11	
Empty breaths	21	550– 360	438.10	58.61	
			Cry Units		
Low	27	2870– 50	444.07	587.41	47
Regular	49	3460–1180	2263.06	558.72	85
Long non-low	15	12050–3600	5092.67	2238.32	88
Other	40	3500– 550	2124.25	866.01	79

TABLE 21–5
SUMMARY OF UNIT DURATION MEASURES FOR INFANT A.G., AGE 14 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	66	4400– 450	1218.93	629.49	
Regular	32	2030– 890	1445.76	291.50	
Long non-low	8	3680–2050	2716.67	613.72	
Other	30	1980– 480	1247.26	406.95	
Empty breaths	16	880– 270	490.00	164.24	
			Cry Units		
Low	66	2620– 50	676.67	530.94	56
Regular	32	1620– 630	1071.36	284.58	74
Long non-low	8	3640–1530	2253.33	740.11	83
Other	30	1660– 80	884.25	406.23	68

TABLE 21–6
SUMMARY OF UNIT DURATION MEASURES FOR INFANT B.H., AGE 3 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	79	1130– 330	542.40	168.92	
Regular	71	1540– 610	962.39	195.75	
Long non-low	18	6500–1780	3383.88	1550.07	
Other	36	1760– 300	998.61	373.27	
Empty breaths	1	240			
			Cry Units		
Low	79	580– 30	212.91	144.25	39
Regular	71	1180– 260	685.77	152.88	71
Long non-low	18	5970–1440	2880.00	1422.10	85
Other	36	1500– 60	694.44	335.56	62

TABLE 21–7

SUMMARY OF UNIT DURATION MEASURES FOR INFANT M.W., AGE 5 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	26	1690– 330	1054.23	332.92	
Regular	69	1620– 870	1134.78	160.27	
Long non-low	66	5160–1640	2596.21	853.74	
Other	17	1590–1100	1424.71	124.53	
Empty breaths	1				
			Cry Units		
Low	26	1200– 80	608.46	299.06	58
Regular	69	1050– 550	706.23	123.90	62
Long non-low	66	4560– 950	2057.58	784.12	79
Other	17	1070– 650	918.82	132.57	64

TABLE 21–8

SUMMARY OF UNIT DURATION MEASURES FOR INFANT R.W., AGE 14 DAYS

Category	*N*	*Range*	$\overline{X}$	*S.D.*	*% of breath unit*
			Breath Units		
Low	25	1270– 590	822.00	164.30	
Regular	155	1410– 670	871.40	154.00	
Long non-low	16	4550–1420	1969.40	803.30	
Other	67	1380– 490	904.80	204.40	
Empty breaths	0				
			Cry Units		
Low	25	850– 290	498.40	153.20	64
Regular	155	1180– 410	595.50	129.40	68
Long non-low	16	4080– 960	1623.10	792.20	82
Other	67	1070– 290	580.30	173.40	64

TABLE 21–9

PERCENTAGE OF UNITS PLACED IN EACH CATEGORY IN THE CRYING OF ALL 8 INFANTS

	MPV	*AG*	*RW*	*MW*	*VW*	*GD*	*BH*	*JG*
Low	40.6	28.5	9.5	14.5	13.0	33.8	38.5	18.0
Regular	22.3	28.5	58.9	38.5	39.8	31.2	34.6	32.0
Long non-low	7.4	4.0	6.1	37.0	28.4	23.1	8.8	10.0
Other	28.7	32.0	25.6	9.5	15.4	7.5	17.6	26.0
Empty breaths	1.0	7.0	0.0	.5	3.2	4.4	.5	14.0

TABLE 21–10

PERCENTAGE OF FEATURES JUDGED TO BE PRESENT IN 4 CRY UNIT CATEGORIES IN THE CRYING OF 4 INFANTS

	FEATURES										
Cry Category	*VFRY*	*Pitch Shift*	*TURB*	*DIM AMP*	*VCHG*	*FEXP*	*RPC*	*VIBR*	*VINSP*	*SIL*	*CLVT*
	Subject: M.P.V., age 7 days										
Low	61.0	11.0	7.0	12.0	29.0	22.0	63.0	10.0	15.0	6.0	12.0
Regular	71.0	7.0	24.0	—	4.0	—	80.0	16.0	51.0	—	9.0
Other	64.0	31.0	16.0	5.0	5.0	71.0	74.0	33.0	14.0	16.0	2.0
Long non-low	93.0	13.0	80.0	13.0	—	7.0	67.0	80.0	13.0	47.0	7.0
	Subject: M.W., age 5 days										
Low	7.7	—	19.2	30.8	38.5	—	80.7	3.8	46.2	46.2	—
Regular	—	—	97.1	—	5.8	—	81.2	1.4	82.6	—	—
Other	29.4	5.9	88.2	23.5	29.4	—	82.4	5.9	76.5	52.9	—
Long non-low	37.1	1.5	93.9	24.2	—	—	72.7	24.2	75.8	83.3	—
	Subject: A.G., age 14 days										
Low	74.2	—	—	6.1	18.1	38.0	48.5	23.0	53.0	33.3	3.0
Regular	68.2	—	69.7	—	3.0	—	80.3	13.6	92.4	—	6.1
Other	77.8	—	44.4	44.4	44.4	22.0	33.3	66.7	33.3	44.4	22.2
Long non-low	78.1	—	11.0	26.0	16.4	—	64.4	26.0	68.5	28.8	12.3
	Subject: B.H., age 3 days										
Low	13.9	2.5	2.5	2.5	82.3	—	35.4	2.5	2.5	3.8	1.2
Regular	9.9	4.2	77.5	—	1.4	—	91.5	—	23.9	—	4.2
Other	19.4	2.8	55.5	8.3	33.3	—	80.6	13.9	16.7	5.5	8.3
Long non-low	88.9	11.1	66.6	22.2	38.9	—	61.1	72.2	11.1	38.9	22.2

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DISCUSSION

BOSMA: Doctors Stark and Nathanson have described an astute and elegant study of infant arousal and vocalization. Their demonstration of the facial and oral gestures and the sounds accompanying the graduations of spontaneous arousal are more meaningful than the earlier studies of cries elicited by imposed pain or other sensory disturbance.[1,2,3] By their extensive, and tedious, observation procedures, they have opportunity to observe the individual infant's patterns of graduation into feeding gestures or into cry, simply or in succession.

The front of mouth gestures of the arousing infant aroused less than in full cry, are remarkably varied in the particulars of their motor patterns and also in their temporal schedules. In the perspectives of this conference, I would wonder whether these varied actions, which are probably developmentally nascent, might not be an appropriate performance substrate for local sensory testing. For instance, could they be modified by application of touch or of a tastant at a critical moment in arousal? I should like to think that these gestures are immediately related to local sensation. During full cry, the front of mouth is relatively insensitive to touch stimulation. In a coincidence which may be meaningful, the motions of the jaw, lips

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[1] Truby, H.M. and Lind, J.: Cry Sounds of the Newborn Infant. *Acta Paed, Scan. Suppl.,* 163:–58, 1965.

[2] Bosma, J.F., Truby, H.M. and Lind, J.: Cry Motions of the Newborn Infant, *Acta Paed. Scan. Suppl.* 163:61–92, 1965.

[3] Wasz-Hockert, O., Lind, J., Vuorenkoski, V., Partanen, T., and Valanne, E.: *The Infant Cry. A Spectrographic and Auditory Analysis.* Lavenham, Suffolk; Spastics Intenat. Medic. Publ. 1968.

and front of tongue are out of schedule with laryngeal function, as indicated by phonation, during full cry.[2]

A final point of emphasis: the mouth has multiple functions, reflecting separate central representations. It is appropriate that we should test oral sensory response in relevance to its several functions.

MALLER: Is the cry pattern for an individual baby stable or consistent over a period of time?

STARK: We do have longitudinal data for 3 of the infants in our sample. This has not yet been analyzed with respect to duration measures. However, from listening to the spontaneous crying obtained from these infants in the third to the eighth week of life, and from looking at some spectrograms of this crying, we can say that many of the auditory and spectrographic features which were characteristic of these infants in the neonatal period persist into the later weeks of life. Also Prechtl et al.,[1] who recorded seven normal infants daily for the first 9 days of life, report that the duration of cries and of the intervals between them were characteristic of the infant and remained so over the period of 9 days, in spite of an overall decrease in the duration of cry and of cry interval.

MALLER: Is there any sex difference in the frequency or pattern of crying that you can determine?

STARK: Our sample of infants is rather a small one from the point of view of answering question about sex differences. We see no evidence thus far of differences in duration of cry units or breath units, nor of cycle duration, which are attributable to sex. It is possible that sex is a factor in fundamental frequency variation. The only suggestion of sex difference we can see at present is that the feature of forceful expulsion of air accompanied by rapid changes in voicing was found in 3 out of the 5 female subjects in our study and in none of the 3 male subjects.

BRADLEY: If you record the cries from a series of infants and then replay these cries randomly to the mother of any one of the infants, could she pick out her baby's cry?

STARK: I have not done this personally but Valanné et al.[1] report such a study and also Formby.[2] Valanné et al. found that, when single cry units were played to mothers from their own and from other newborn infants, about one-third of the mothers correctly discriminated their own and other infants' cries. Formby, on the other hand, found that when longer portions of crying were presented to mothers and they were asked to select their own infant's crying from a set of 5 cry recordings, about two-thirds of the mothers were successful. In both studies, the performance of the mother appeared to improve after the infant's 48th hour of life. The results of the 2 studies suggest that the mother's recognition of her own infant may be improved if you give her longer portions of crying to listen to rather than single cry bursts only. It might be worthwhile to test this finding more carefully. Personally, I can recognize the crying of all of the infants we have studied, but I have no doubt that I do this in an analytical way and not as the mothers do it.

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[1] Prechtl, H.F.R., Theorell, K., Gramsbergen, A., and Lind, J. A statistical analysis of cry patterns in normal and abnormal newborn infants. *Develop. Med. Child Neurol.*, 11:142–152, 1969.

[1] Valanné, R., Vuorenkoski, V., Partanen, T.J., Lind, J., Wasz-Hockert, O. The ability of human mothers to identify the hunger cry signals of their own newborn infants during the lying-in period. *Experientia*, 23:768–769, 1967.

[2] Formby, D., Maternal recognition of infant's cry. *Develop. Med. Child Neurol.*, 9:293–298, 1967.

BRADLEY: If a lamb, which has been taken from a ewe for a period of time, is put back with the flock, the lamb bleats and runs to the nearest ewe. The lamb does not appear to recognise its mother. However, the ewe seems to recognise her lamb by its bleats and actively moves toward her lamb. It is the ewe that finds the lamb, not the lamb which finds the ewe.

WEIFFENBACH: In spite of the importance of distance receptors in subsequent recognition, the initial postnatal identification of the lamb by the ewe appears to depend on a type of contact in which the olfactory and gustatory system are primary.

I understand that the ewe will spend 20–30 minutes licking and cleaning her own lamb or the first of any recently born lambs presented to her within a period of several hours after she has given birth. It has been suggested that an extremely rapid type of learning analogous to imprinting, takes place at this time and supports the ewe's later rejection of lambs that she did not lick during this critical period.[1] Audition and vision are good candidates for the effective sensory channels for infant identification but it looks like oral behavior and, presumably oral sensation, play an important role in the formation of the ewe's relatively exclusive attachment relation with a lamb.

STARK: On the other hand, the California sea-lion[2] produces a category of call which shows consistency within individuals and variability across individuals. These calls are used by both the mother and the pup in finding one another in the rookery.

ZLATIN: Were you able to get any judgments of these cries from the mothers as to the state of the infant or how they interpreted the type of cry?

STARK: The mothers who were present at the time judged their infants to be hungry and fed them as soon as I gave them the signal that the recording was at an end. It is not possible, of course, to say how they arrived at that judgement. They might have used cues from the infants' non-vocal behavior, active searching with the mouth, sucking and rooting, from their knowledge of the infant's immediate past history, i.e., that he had not been fed for 4 hours or so, or features of the cry itself such as the forceful expulsion of air feature, or some combination of these. As far as the forceful expulsion of air feature itself is concerned, it does not elicit alarm in the mother in the same way that certain features of the pain cry elicit alarm (cf Wolff, 1969).

STEINER: I was impressed by the phenomenological description of the vocal manifestation of the spontaneous cry. Do you have any idea about the precursors of the spontaneous cry? Do you have any physiological indicator of preparation or of anticipation of spontaneous cry?

STARK: Unfortunately, not yet. I do think there are respiratory changes, though, that herald this.

STEINER: What about "hunger", or sugar level, or any other biochemical value which can indicate hunger?

STARK: It would be nice to have such indicators although in the very process of obtaining them, we might elicit a pain cry. Thus we would have to design the experiment so that the collection of blood samples and recording of the cry were done under identical circumstances, but not at the same time.

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MALLER: Is there any information available on the relationship between hunger and spontaneous crying?

STARK: Wasz-Hockert et al.[1] found that the hunger cry, i.e., steady crying produced 4 hours ± 20 minutes after the previous meal when, all the motives for discomfort other than hunger had been excluded as far as possible, differed from other types of cry in certain ways. For example, over 2/3 of the hunger cry signals they recorded had a rising-falling form, and the maximum pitch was lower than for the pain-elicited cry. Glottal plosives, i.e., the feature we have called forceful expulsion of air, was a characteristic feature which became commoner after the first month. Pitch shift and sub-harmonic break were rarely found.

ZLATIN: Did the differences in initiation of cry relate to the age of the infant and/or his state prior to administering the stimulus?

STARK: We think there is too much individual variation in our sample and perhaps too small an age range for us to be able to say anything about the effects of age upon differences in initiation of cry at this point. As pointed out above, Wasz-Hockert et al.[1] found glottal plosives, which we have found to be used in initiatory cry, increased in frequency with the age of the infant. So possibly this mechanism is used more often to initiate cry by older than by younger infants. With respect to state, I would say that the infants who were more fully aroused appeared to initiate cry with greater ease. Also, as indicated above, their crying was more sustained, so that there were fewer occasions upon which they used initiatory sounds. The judgement of degree of arousal was made not only on the basis of amount of crying, of course, but on the basis of amount of non-cry activity, e.g., arm and leg movements which were taking place and on facial expression.

JOHNSON: Have you studied the respiratory physiological correlates of crying?

STARK: We have not made measurements of blood gases and cannot at this time say what their values are during the cry episode. However, the average duration of the breath unit during spontaneous cry across all infants was 1480 msec. This is the equivalent of 40.5 breaths per minute, which is not very different from the mean respiration rate during non-cry intervals reported by Proctor and Hardy[1] and others. The main difference between cry and non-cry respiration, as the work of Bosma, Truby and Lind[2] indicates, is in the relative duration of the expiratory and inspiratory portions of the breath unit, i.e., the expiratory portion is lengthened and the inspiratory portion shortened in cry as compared with non-cry. However, some cries which are of greater than average duration or are accompanied by greater airflow during the expiratory phase may induce hypoxia more readily than others. We cannot, in the absence of information about p02 and PH levels, look for any consistent effect of hypoxia due to crying upon subsequent crying behavior, although such effects may be present. They might take the form of increased intake of air during the inspiratory phase or of the replacement of two separate intakes of air for the more usual single intake (two intakes are reflected in what we have called an empty breath, i.e., a breath unit in mid-cycle in which no vocalization occurs).

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ZLATIN: It was most interesting to observe at times the synergistic movements of the upper extremities upon initiation of cry. These appeared to be especially prominent with elevation of the mandible. Did you note any variations in head or body position that may have been reflected in differential initiation of cry behavior?

STARK: We have become aware of the different styles of initiation of cry as this study has progressed. It is our impression that variations in head and body position were not associated with these different styles and this is borne out by examination of the cinephotographic data we have obtained on one infant. We also have the impression, however, that those infants whose initiating cries were long and stressful showed greater immobility of head, face and limbs during cry initiation than those who began to cry less effortlessly.

WEIFFENBACH: I would like to ask about the symmetry and asymmetry of tongue motions. Most of the tongue movements that I saw in your film were laterally symmetrical in the sense that elevation of the tongue was not accompanied by movements away from the midline. However, I am interested in the situation where displacements from midline do occur but none the less symmetry is preserved at the level of pattern by the fact that a lateral displacement to one side is balanced by a subsequent displacement to the other. Your films show infants displaying just such a symmetrical pattern of lateral displacements in their arm movements. Occasionally, while attempting to open the mouth of a fussy newborn I see a sequence of alternating lateral displacements of the tongue. Do the tongue movements of your undisturbed infants show this type of symmetrical pattern of lateral movements?

STARK: No, I have not seen lateral displacement of the tongue to one side, followed by subsequent displacement to the other side in the spontaneous crying of newborn infants. Of course it is hard to make these judgements in the absence of a visual record, and it may be that this does take place and I have missed it. As I indicated, we have seen one side of the tongue move differently from the other without displacement from the midline, and I have seen the lips move differently on one side than on the other in crying. These might be considered examples of asymmetry. I have also seen another kind of asymmetry, i.e., elevation and depression of the tongue accompanied by deviation from the midline and not followed by complementary deviation to the other side, in much older infants. This seemed to be a voluntary action in their case.

KAWAMURA: Is there any possibility of an effect of posture on the cry. Did you record the cry only in the prone position?

STARK: We recorded crying only when the infant was in the supine or side-lying posture. I could not change the infant's posture without handling him, and, of course, when handled the infant may stop crying or cry less stress-fully in a manner more like that of non-cry vocalization, especially if preparations are being made to give him the breast. It would be hard then to assess the effects of posture per se.

KAVANAGH: Dr. Stark, have you recorded the cry of an infant who has later been diagnosed as congenitally deaf or of an infant who has later been identified as having been emotionally deprived? In the latter case, I was thinking particularly about the children who Blizzard of the Johns Hopkins Medical School has described as children with extreme psycho-social deprivation.

STARK: No. I have tried to locate very young infants suspected of having a hearing loss, but in every case the infants referred to me have later turned out to have normal hearing or very moderate losses of hearing. Perhaps you were thinking of Jones'[4] finding of greater variability of fundamental frequency in the crying of hearing-impaired children?

KAVANAGH: I was prompted to ask the question by the observations that the infant's cry (or lack of cry) is affected by the amount and kind of handling he receives from his mother. Also by his own cry and the cry of other infants.

STARK: Yes, that's true. Aldrich et al.[1] found that crying was most frequent in the newborn nursery at those times when few nurses were available to attend to the infants and less frequent when the infant was home with his mother than in the newborn nursery. Also Bell and Ainsworth[2] have shown that the more responsive the mother is to her infant's crying, the greater the likelihood that crying will decrease over the first year of life and that other communicative signals will become operative. I am not so certain of the effects of the infants' own auditory feedback upon crying although, judging from the effects of delayed auditory feedback upon cry, these effects are present.[3, 4]

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Chapter 22

MOTOR ABNORMALITIES IN INFANT DOGS INDUCED BY INTERMITTENT ORAL STIMULATION

WALTER C. STANLEY

During intermittent feeding and other oral stimulations infant dogs may display a syndrome of maladaptation consisting of a dystonia and stereotyped dyskinesia of lingual and postural responses involving tongue, mouth, neck, limbs, and trunk.[16,17,18] Psychologically, these motor abnormalities are of interest because they represent developmentally early forms of adjunctive behavior that occur in infant dogs in operant conditioning situations.[16] Neurologically, the motor abnormalities appear related to "tongue sucking" in human infants[11] and to clinically observed syndromes of drug-induced oral dystonias and dyskinesias in human adults.[1,2,3,7,8]

There are two highly distinctive patterns or variants of the maladaptive behavioral syndrome: tongue-on-roof of mouth (TOR) and tongue-on-floor of mouth (TOF). The purpose of this Chapter is to describe the behavioral contexts in which the maladaptive TOR-TOF states have been observed; detail their sequential response components; present data indicative of the places during operant conditioning sessions where they are most likely to occur; and summarize experimental findings on the kinds of intermittent oral stimulation which are effective in inducing the lingual components of both TOR and TOF.

BEHAVIORAL CONTEXTS IN WHICH MALADAPTIVE TOR-TOF STATES OCCUR

Systematic recorded observations of the maladaptive behavioral states have been made in operant key lifting situations where the reinforcer was milk contingent on nipple sucking, and in situations where the experimenter repeatedly stimulated a fixed amount of sucking via placement of the finger or artificial nipple in the mouth of the puppy.[16,17,18] Prior to these systematic studies my coworkers and I had observed (but not systematically recorded, described, or reported) the TOR lingual pattern in operant nipple sucking situations,[13] discriminated instrumental nipple approach situations,[13,19] and hand feeding situations with an artificial nipple where neonatal puppies were being maintained completely away from the dam.

All of the foregoing situations have in common either interrupted or clearly intermittent oral stimulation under a high level of food deprivation, i.e., under

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The author expresses his gratitude to Gertrude Nicholson, medical artist, who traced and drew material for Figures 1 and 2, and to E. Faye Bacon, Stephen N. Lee, and Leslie D. Heiple who assisted in the research.

conditions of strong instigation to feed. Such situations both descriptively and theoretically are regarded by many as intensely frustrating. It would be an error, however, to ascribe the behavioral state to frustration or to intense emotional excitement and thus to forego its objective investigation. Neither frustration nor emotional excitement are *directly* measurable properties of an organism, of the environment, or of the interaction of organism and environment. In contrast, interrupted and intermittent stimulations are directly measurable and manipulable as are other properties of the past and current history of the animal. Despite the apparent descriptive applicability of frustration and emotional excitement, therefore, these terms shall be omitted from further consideration in this Chapter because they are heuristically barren unless assigned surplus meanings based on inferences from behavior.

Initial Observations of TOR States

For approximately eight years (1963–70), TOR in our laboratory was primarily a protocol abbreviation for the neonatal puppy's behavior that precluded normal milk-obtaining via suction from the artificial nipple in our multiple feeding apparatus (Fig. 8–9 in Stanley[15]). During TOR the puppy was attached to the nipple, emitted observable sucking movements of the mandible but generated no negative pressure recordable either in analog form by an oscillograph or in discrete event form by a cumulative response recorder. Close examination of the puppy and its partial removal from the nipple revealed that its tongue was above, rather than below, the nipple. The failure to generate negative pressure was clearly caused by this aberrant positioning of the tongue against the roof of the mouth and above the nipple rather than below the nipple with lateral edges curled part way around the nipple to form an airtight seal between the tip of the nipple and the mouth of the puppy.

Inefficient milk obtainers who gained little or no body weight each day spent considerable time with their mouths closed while they pushed against the nipple and/or the hole (1.6 cm. in diameter) through which the nipple protruded into the apparatus. To stimulate such a puppy to suck, the experimenter pried its mouth open, sometimes only with real effort, inserted his finger in the normal position above the tongue, then gently led the puppy to crawl and become normally attached to the nipple by slipping the puppy's mouth around the nipple as the finger was simultaneously being withdrawn from its mouth. If this complex procedure was not followed, a puppy might (a) push against the nipple and nipple hole for several minutes until the session ended by withdrawal of nipple; (b) repeatedly slip onto the nipple with TOR maintained and the nipple below rather than above the tongue; (c) assume a generally opisthotonic posture with head raised, mouth closed, and extensor rigidity of fore limbs if the experimenter grasped its neck and shoulders and pushed its snout against the tip of the nipple; or (d) latch on the nipple normally.

In general, the foregoing description is also valid for discriminated instrumental conditioning situations where a milk-containing nipple was available only when specific tactual cues were present. The complex procedure of prying open the mouth, inserting the finger, then inserting the nipple while withdrawing the finger was also sometimes necessary to initiate normal sucking in a lap-held puppy being fed via Playtex nipple and nurser. Over a half dozen technicians and research assistants learned to recognize this maladaptive TOR sucking pattern, to note it in protocols, and to correct it, i.e., stimulate normal sucking, by various techniques. For years in our laboratory, it was regarded largely as a nuisance because it interfered with accurate measurement of puppies' self-feeding in automated apparatuses, led to unplanned undernourishment of puppies, and introduced time delays between behavior and milk-obtaining detrimental to efficient acquisition and maintenance of learned behavior based on reinforcement with milk.

Discovery of Adjunctive TOR and Adjunctive TOF States

Under certain conditions, adjunctive behavior emerges in operant conditioning situations even though it is not required for reinforcer presentation.[6,10] Typically, the adjunctive behavior occurs primarily if not exclusively immediately after the termination of each reinforcement; it only rarely occurs once the subject has begun a run of operant responses which will eventuate in reinforcer presentation.

We conducted the first operant key lifting experiment on a litter of infant beagle dogs in 1970.[14] The key lifting performance of one of the six subjects in the litter became increasingly disturbed by frequent occurrence of adjunctive TORs and related motor abnormalities (Fig. 18–1, 18–2 and 18–3, Bea 544♂ in Stanley.)[16] The excessively maladaptive adjunctive behavior of this puppy made us realize that we had discovered a genuinely new behavioral phenomenon.

Despite our 8-year experience with canine infant TORs in other behavioral contexts, we initially failed to recognize the TORs as they occurred in the new context of the operant key lifting situation. At first, our protocol notes of the behavior of 544♂ were simply erroneous. The puppy was "seen" as "licking the wall or floor." But subsequent careful observations of the same puppy in the same postures confirmed the presence of TORs. We had misinterpreted suck-like movements of the mandible typically correlated with TOR as licking characteristic of older puppies.

As our operant research in infant beagles continued, we found additional puppies who displayed markedly maladaptive adjunctive TORs, noted that even apparently undisturbed puppies had occasional mild TORs, and discovered a second major pattern of highly maladaptive behavior, the adjunctive TOF state.

The Operant Key Lifting Situation as a Context for Maladaptive Adjunctive TOR-TOF States

The maladaptive nature of the TOR-TOF abnormalities was remarkably striking in the operant key lifting situation. Puppies who had behaved "knowledgeably" suddenly lost their adaptive behavior and became completely ineffectual. For this reason a resume of the operant apparatus and procedure is necessary here, even though apparatus, procedure, and selected stylized postures have already been described in some detail.[16]

In the operant key lifting situation puppies were required to lift a key with the snout one or more times and thereby activate a nipple insertor-retractor. The nipple entered the experimental chamber through a hole (Fig. 8–24 in Stanley).[15] Puppies were required to latch on the nipple, suck, and thereby activate milk injection into their mouths via the action of a peristaltic pump. The nipple withdrew automatically through the hole in the apparatus after puppies had obtained a scheduled amount of milk, usually five 0.1 ml. squirts of milk contingent on each, or every other, suck. Then puppies had to lift the key again one or more times, latch on the nipple, suck until the nipple withdrew, then lift the key again, etc., until a predetermined session duration or session milk quota was reached. Thus far, complete patterns of TOR-TOF motor abnormalities have been observed only in the operant key lifting situation.

SEQUENTIAL LINGUAL AND POSTURAL COMPONENTS OF TOR-TOF STATES

Sequential response components of TOR-TOF states in the operant situation consist of the following: (a) sustained tongue position—TOR or TOF: (b) disappearance of normally directed and component movements of mouth, head, limbs, and trunk; (c) apparently rigid and stylized body posturing variously accompanied by stereotypic and repetitive movements of mandible, head, limbs, trunk; and (d) sudden disappearance of motor abnormalities and concomitant reappearance of normal lingual mobility and feeding movements.

To depict the lingual, oral, and postural components of the TOR-TOF states as these occurred in the operant key lifting situation, we video-taped pictures of four 12-day-old littermate purebred beagle puppies while they were working in the apparatus. One puppy was male, three female. At birth the male weighed 330 and the females 290, 347, and 376 grams; at five days of age they were permanently separated from the dam and weighed, respectively, 490, 484, 522 and 564 grams; from 5–12 days of age they received training sessions in operant key lifting, the reinforcer being milk via sucking on a milk containing nipple. When video-taped (Session 29, age 12 days), the puppies weighed 491, 486, 514 and 578 grams and were required to lift the key eight times to produce each nipple insertion.

The video-taped pictures were studied by a medical artist and then overlapping drawings were made of the sequential components of the two variants of the maladaptive syndrome. The aim of the drawings was not to represent the performance of a single animal during a single episode. Rather, it was to illustrate in full and in proper sequence all the major response components that could be abstracted from viewing repeatedly the performances of all four puppies with action at normal speed and stopped to permit tracing in detail of each component response.

Lingual, oral, and postural response components of the TOR variant (Fig. 22–1) began right after the nipple was fully withdrawn from the puppy's mouth (Fig. 22–1, Frame 2). The tongue's mobility was arrested and the tongue assumed a fixed position against the roof of the puppy's mouth. There occurred suck-like movements at normal rate (ca. 1.5—2.5/sec.) or at abnormally slowed rate (ca. half the normal rate). Forelegs, head, and upper trunk were extended and a generally prone position was maintained. The forward head position alternated with a downward head position. The puppy would slowly rotate in

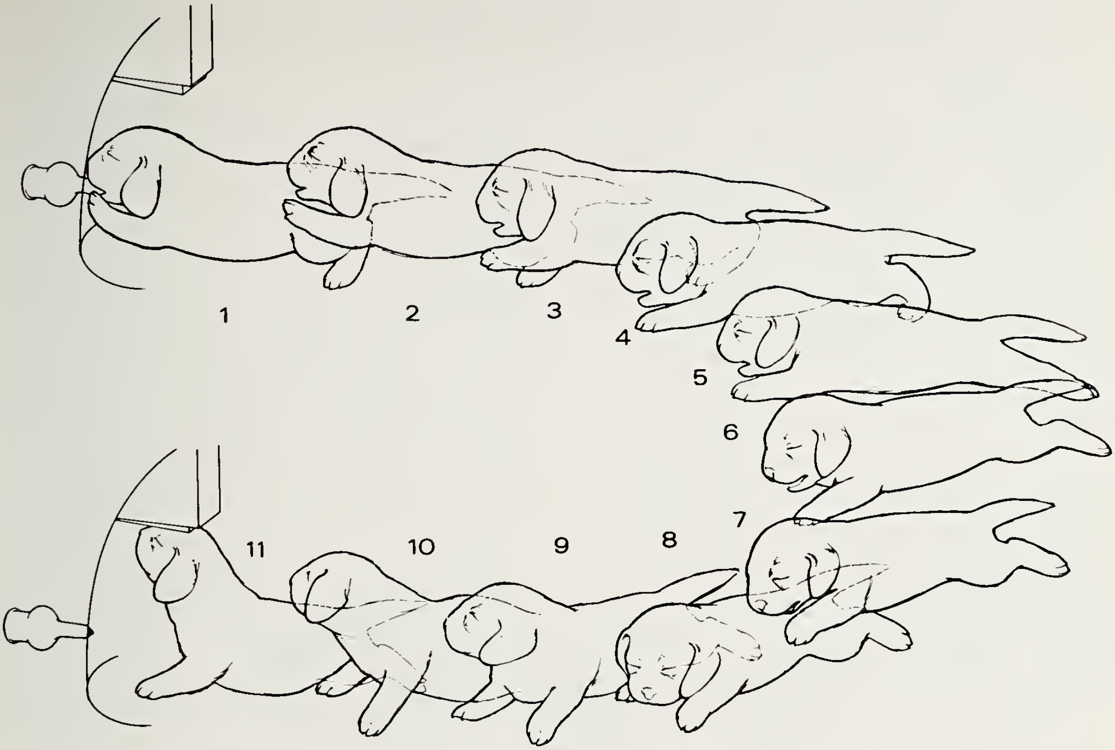

Figure 22–1. Sequential postures of the TOR (tongue-on-roof of mouth) pattern of the maladaptive adjunctive behavioral state. Frame 1, the nipple is being retracted; 2, the tongue assumes its immobile position against the roof of the mouth; 3-8, the puppy progressively displays stylized postures associated with TOR, i.e., head lowered to the floor, front paws rigidly extended and mandible moving rhythmically and stereotypically; 9-11, the tongue becomes suddenly mobile and the puppy resumes normal posture and returns to key lifting.

the horizontal plane, clockwise or counter-clockwise (Fig. 22–1, Frames 3–8). Termination of the state consisted of abrupt return of lingual mobility and of normal conditoned and unconditioned "directed" and "searching" feeding behavior patterns normal for dogs of this age (Fig. 22–1, Frames 9–11).

The TOF variant began in the same fashion as the TOR variant (Fig. 22–2, Frame 2). After full withdrawal of nipple from the puppy's mouth, lingual mobility was arrested and the tongue assumed a fixed position against the floor of the mouth. An older puppy, i.e., one approaching, or in, the third week of life, can sit, stand, and initiate some walking. For these reasons, apparently, the puppy's withdrawal-from-nipple-hole behavior (Fig. 22–2, Frames 3–5) automatically led the puppy to assume partial or full sitting position, but with head thrust downward in a median position without rotation, a position reminiscent of the profile of a sitting buffalo; hence the descriptive phrase, "buffalo stance." The Forelegs were extended and thrusted alternately and stiffly against the supporting surface (Fig. 22–2, Frames 5–6, 8). Rear legs were not extended. The mouth was open and suck-like movements occurred (Fig. 22–2, Frames 4–8). The head jerked downward or sideward or rotated jerkily

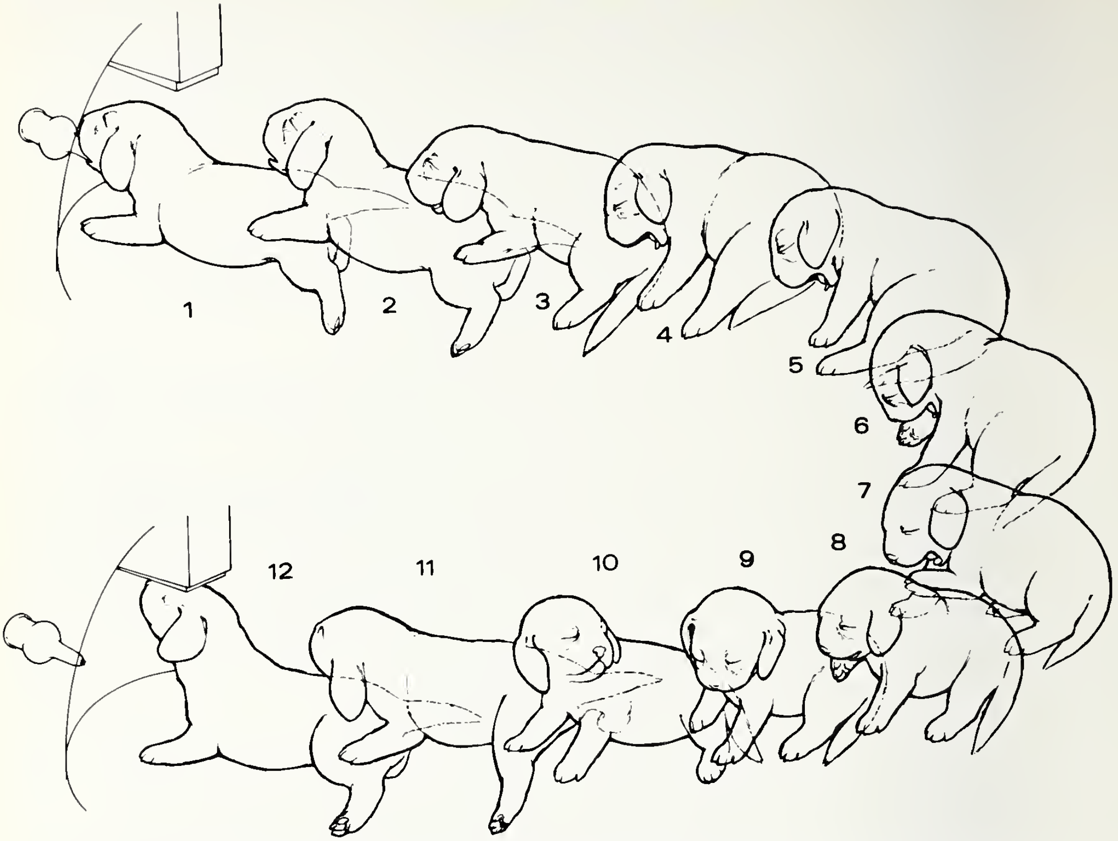

Figure 22–2. Sequential postures of the TOF (tongue-on-floor of mouth) pattern of the maladaptive adjunctive behavioral state. Frame 1, the nipple is being retracted; 2, the tongue assumes its immobile position against the floor of the mouth; 3-8 the puppy displays the stylized "buffalo stance" associated with TOF in which it raises its forequarters and moves backward with rigid and treading foreleg action, lowers its head, opens its mouth wide and exhibits rhythmical and stereotypic movements of the jaw; 9-12, the tongue becomes suddenly mobile and the puppy resumes normal posture and returns to key lifting.

around the rostral-caudal axis, leading the puppy to fall on its side or into a sideward roll that terminated in a normal stance on all four legs (not depicted in Fig. 2, but rollovers tended to start from positions shown in Fig. 22–2, Frames 4–8). Outcomes of rollovers were variable. A TOF state might be terminated temporarily, permanently, or left unchanged, the puppy resuming a sitting or a standing buffalo stance and then repeating the pattern of responses it made before the rollover. If a sideward jerk terminated the state (Fig. 22–2, Frame 8), there followed a resumption of conditioned and unconditioned directed and searching feeding behaviors normal for a puppy of this age with prior operant conditioning (Fig. 22–2, Frames 9–12).

CONDITIONS AFFECTING OCCURRENCE OF TOR-TOF STATES WITHIN OPERANT SESSIONS

Up to this point the maladaptive TOR-TOF syndrome has been described by its appearance in operant conditioning experiments. These experiments were originally designed to study quantitative properties of operant key lifting, not quantitative properties of maladaptive adjunctive behavior. In the following experiment, key lifting was measured as before but the primary focus was on careful observation and recording of the onset and duration of TOR and TOF responses.

Onset and duration were indicated by manually marking chart paper moving at 1 cm. per min. The marks were later measured to nearest .5 mm. which is equivalent to duration to the nearest 3 sec. or .05 min. To preclude possible severe undernourishment and loss of body weight in puppies, TOR-TOF states were usually broken up by the experimenter if they lasted 2 min. Exceptions, when made, occurred on the last session of the day because supplementary feeding with Playtex nipple and nurser could be easily scheduled at that time without disrupting the within day experimental schedule.

Experimental manipulations and data analyses were carried out to assess the TOR and TOF states as follows: time puppies spent in TOR-TOF states while in the operant apparatus; ordinal number of milk-containing nipple insertion-retraction within sessions; re-

TABLE 22–1

BODY WEIGHT, TIME SPENT IN OPERANT APPARATUS, AND TOTAL AND PERCENTAGE OF TIME SPENT IN THE TOR–TOF STATE WHILE IN APPARATUS IN FOUR LITTERMATE PUREBRED BEAGLE PUPPIES

	Body Weight (g) at:			*Time (min.) in:*		
Subject No. & Sex	*Birth*	*Sess. 5, Age 11 days*	*Sess. 48, Age 21 days*	*Apparatus*	*TOR–TOF State*	*%*
1072♀	279	452	588	1127.7	383.8	34.0
			596[1]	1181.3[2]	403.3[2]	34.1[2]
1070♂	386	557	734	855.0	77.5	9.1
			744[1]	908.1[2]	88.0[2]	9.7[2]
1071♂	329	608	753	849.2	49.1	5.8
1073♀	347	516	664	842.5	7.0	0.8

When 6 days old, puppies were removed permanently from the dam and received two feedings via Playtex nipple and nurser, each to quota of 33 ml/kg body weight. Thereafter they received four feedings per day via Playtex nipple through 9 days of age; then via performance in the operant key lift apparatus from 10–21 days of age and supplementary feeding as necessary. Puppies 1072♀ and 1970♂ received 3 additional sessions when 22 days old. See Fig. 3 for milk quota changes. In this litter the maladaptive syndrome was primarily TOR with TOF being observed only briefly and occasionally.

[1] Values for Session 51, Age 22 days.

[2] Values based on data from Sessions 6–51, other values in these columns are based on Sessions 6–48. Sessions 1–5 were omitted because these were largely training sessions with considerable help from the experimenter.

turn late in training to a continuous reinforcement (CRF) schedule wherein each key lift is followed by milk-containing nipple insertion after considerable prior training with a fixed ratio (FR) of 9 key lifts required for milk-containing nipple insertion; and increasing the level of food deprivation by reducing the session milk quota.

Time Spent in TOR-TOF States

Data on time spent in TOR-TOF states appear in Table 22–1, along with pertinent information concerning the infant beagle subjects and their training. The maladaptive syndrome in this litter was predominantly TOR with TOF being observed only briefly and occasionally.

The table shows that the lightest animal (1072 ♀) showed the greatest amount of maladaptive adjunctive behavior (34% over Sessions 5–48) while the two heavy animals (1070 ♂ and 1071 ♂) showed intermediate amounts (9.1 and 5.8%) and the second lightest animal (1073 ♀) showed the least amount (0.8%).

Effect of Ordinal Number of Nipple Insertion-Retraction

Almost without exception the TOR-TOF state occurred after nipple with-

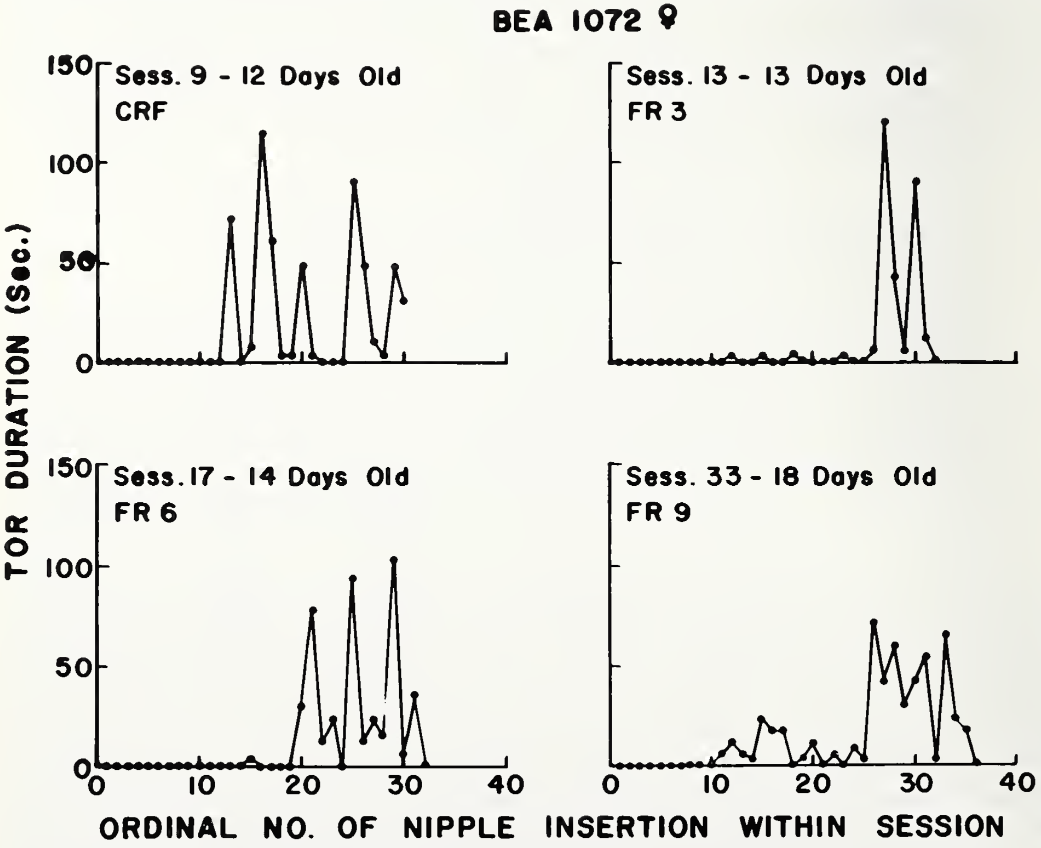

Figure 22–3. Duration of TOR responses plotted as a function of ordinal number of nipple insertion-retraction within operant sessions 9, 13, 17, and 33. Abbreviations are: *CRF*, continuous reinforcement, i.e., one key lift required for nipple insertion into apparatus; *FR* 3, fixed ratio 3, i.e., three key lifts required for one nipple insertion; etc.

drawal and before the puppy started key lifting after a post-reinforcement break in operant performance. Almost without exception, the first occurrence of the TOR-TOF state came after a nipple insertion-retraction other than the first. Indeed, it typically came considerably later in the session as shown in Figure 22–3.

The trend over the sessions depicted is in line with prior observations and conclusions[16] in showing that frequency of TOR-TOF occurrence was greater with FR 9 than with CRF, FR 3, and FR 6, but apparently out of line in showing that frequency of long duration TOR-TOF responses (greater than 100 sec.) was smallest in the FR 9 session shown.

Effect of Return to Continuous Reinforcement (CRF)

In Figure 22–4 it can be seen that a return to CRF did not have an ameliorating effect on TOR-TOF occurrence during the first session (39) in which it was reintroduced. Pronounced ameliorating effects were evident, however, in the second and third sessions (40 and 41) of CRF reintroduction. When FR 9 was reintroduced (Session 42), maladaptive adjunctive behavior returned.

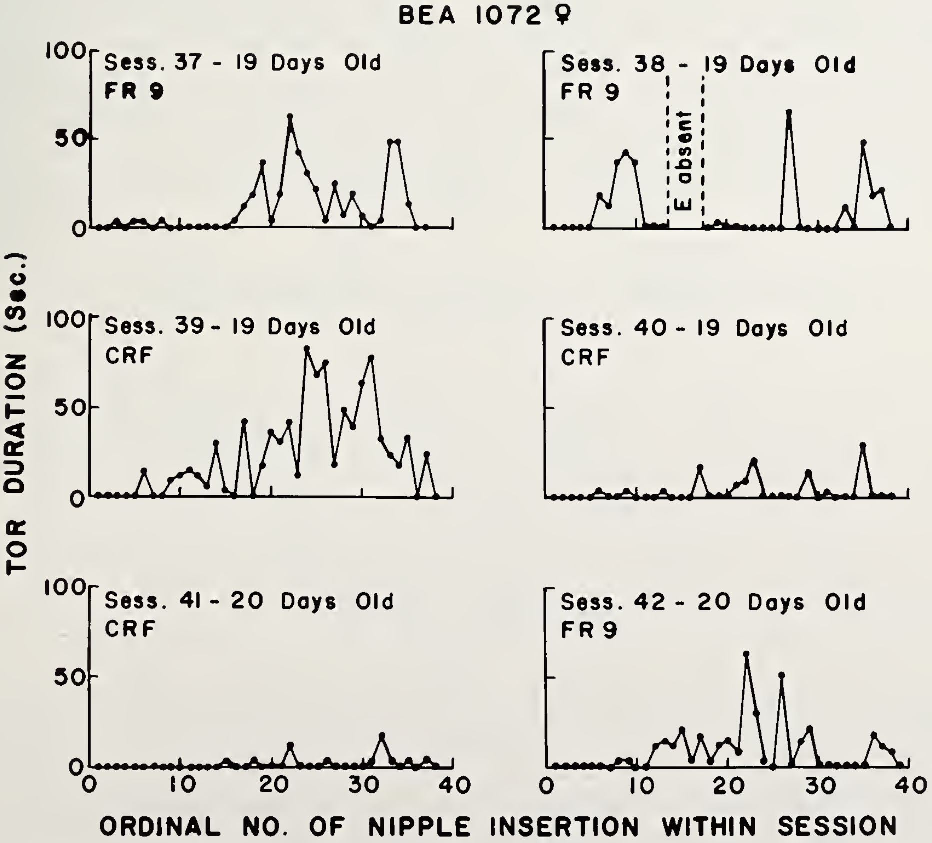

Figure 22–4. Duration of TOR responses plotted as a function of ordinal number of nipple insertion-retraction within operant sessions 37-42. During Session 38, information was missing because the experimenter had to leave the experimental room (*E absent*). Other abbreviations are the same as in Figure 3.

Effect of Increasing Level of Food Deprivation and Other Factors

Previously we had observed that light weight animals in a litter might be especially prone to succumb to TOR-TOF states of long duration. This was the case in the present experiment (Table 22–1). It was somewhat surprising, therefore, that we obtained no convincing evidence that increased level of food deprivation augmented maladaptive TOR-TOF. For example, if food deprivation had augmented TOR-TOF, then key lifting performance should have been impaired. But Figure 22–5 shows that reduced milk quotas ($Q = 25$ ml/kg for Subject 1070♂ and 1073♀ and $Q = 17$ ml/kg for Subject 1071♂) had no perceptible detrimental effect on ensuing key lifting behavior.

Subject 1073♀ eventually did show unstable performance, but the instability came 20 sessions after the first quota reduction and also eight sessions after full quota ($Q = 33$ ml/kg) had been restored. Clearly, the behavioral instabil-

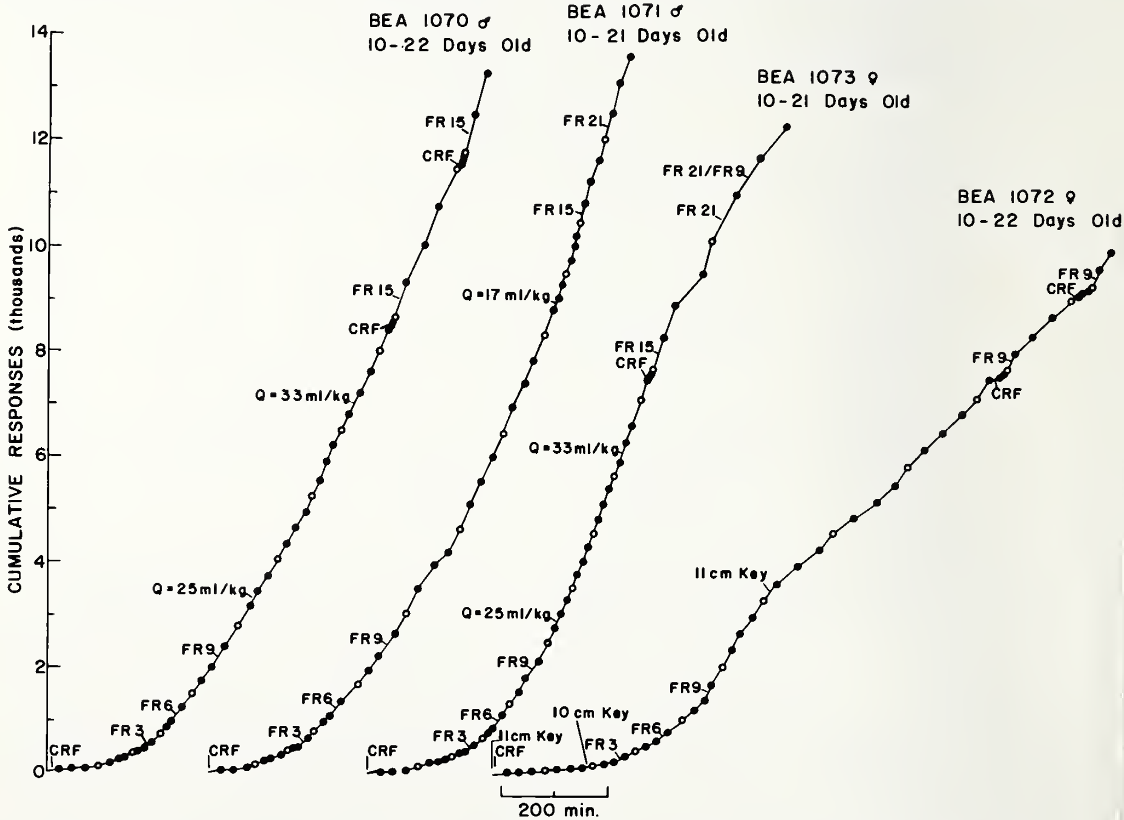

Figure 22–5. Number of key lifts cumulated over sessions and plotted (with open circles representing the first session of each day and closed circles the remaining sessions) as a function of cumulated time in the operant keylift apparatus beginning with the second session and ending with the 51st session (puppies 1070♂ and 1072♀) or the 48th session (puppies 1071♂ and 1973♀). To form the time baseline, 5 sec. was subtracted for each nipple insertion because this was the minimum time required for an efficient puppy to latch on the nipple and obtain five milks on an FR 2 schedule. Abbreviations are: *CRF*, continuous reinforcement; *FR*, fixed ratio; *Q*, quota of milk per session (all puppies started with $Q = 33$ ml/kg body weight); *cm. key*, height of key from floor of apparatus.

ity cannot be ascribed to food deprivation. Instead, this Subject's instability and reduced rate of performance near the end of its training were almost certainly due to increasing work requirement from 9 key lifts per nipple insertion (FR 9) to 15 (FR 15) and then 21 (FR 21) key lifts per nipple insertion. Such work requirements are like extinction procedures in that much work is performed but the animal receives no reinforcement. Omission of a positive reinforcer is typically accompanied for a time by emotional responding. This was true for Subject 1073♀. This puppy vocalized loudly, oriented toward the experimenter rather than the key, then gradually became quiet and went to sleep.

It is thus clear that none of the performances depicted in Figure 22–5 indicate that reduced food quotas increased maladaptive TOR-TOF states. Nevertheless we cannot conclude that food deprivation and correlated increased hunger cannot augment maladaptive TOR-TOF states. It may be that food deprivation must be present before proficiency in operant responding is attained in order to affect TOR-TOF behavior. When puppies are not proficient or are small and incoordinated, they may be prone to excessive TOR-TOF behavior because they cannot reach the key easily or consistently with their noses. Such incapacity could lead to intermittent or occasional reinforcement because many specific responses would miss the key. In this connection note that the key height had to be lowered for puppy 1072♀ (Fig. 22–5). However, when puppies are skillful, food deprivation and correlated hunger might enhance operant key lifting and other behavior. The other behavior could be "collateral" or "interim" activities which "mediate" successful performance, as they do in adults.[9,12]

STIMULATION OF THE LINGUAL COMPONENTS OF THE MOTOR ABNORMALITIES

Our findings are consistent with the conclusion that maladaptive TOR-TOF behaviors in infant dogs in operant feeding situations are examples of intermittent-feeding-schedule-induced adjunctive behavior.[15] If this conclusion is valid, the TOR-TOF behaviors in infant dogs are analogous to schedule-induced polydipsia in adult rats as first described by Falk,[4] and to other forms of adjunctive behavior in other animals.[5,6,10] Neurologically, however, the oral manifestations appear similar to human dystonias and dyskinesias.[1,2,3,7,8] One would expect on both psychological and neurological grounds, therefore, that intermittent oral stimulation alone might influence the topography and probability of occurrence of specific components of the maladaptive state. Accordingly, we carried out a group-comparison experiment to determine the relationships between TOR-TOF patterns of response and three types of oral stimulation, age and sex of infant dogs, and Day 1 vs. Day 2 of testing.[17]

Stimulus objects used for repeated oral stimulation were: (a) finger below (FB) in which the index finger was placed palmar side up below the tongue; (b) finger above (FA) in which the index finger was placed palmar side down above the tongue; and (c) milk-containing nipple (MN) in which a milk-containing Playtex nipple was placed in the mouth above the tongue.

Thirty purebred beagle puppies were permanently removed from their dams at 3–7 days of age and 30 at 14–16 days of age. Within each session the same five male and five female puppies in each age range were stimulated up to 10 times to suck for 3 sec. with the same type of oral stimulus. The stimulations came 15 sec. after a preceding TOR-TOF response had terminated and the tongue had become normally mobile, 15 sec. after a response was terminated by the experimenter at 2 min., or 15 sec. after prior stimulation had ended if no response was observed. Each session ended after 10 stimulations (trials) or after a puppy failed to suck for 20 min. Three tests sessions per day were conducted on the two successive days following removal of puppies from dams. One hour after each session, each puppy was fed milk via Playtex nipple and nurser to a total intake quota of 50 ml/kg body weight.

The TOR was the most frequent and the most enduring response to FB; it was an occasional and brief response to FA and MN (Fig. 22–6). TOR differences resulting from conditions of stimulation were greater in younger than older pup-

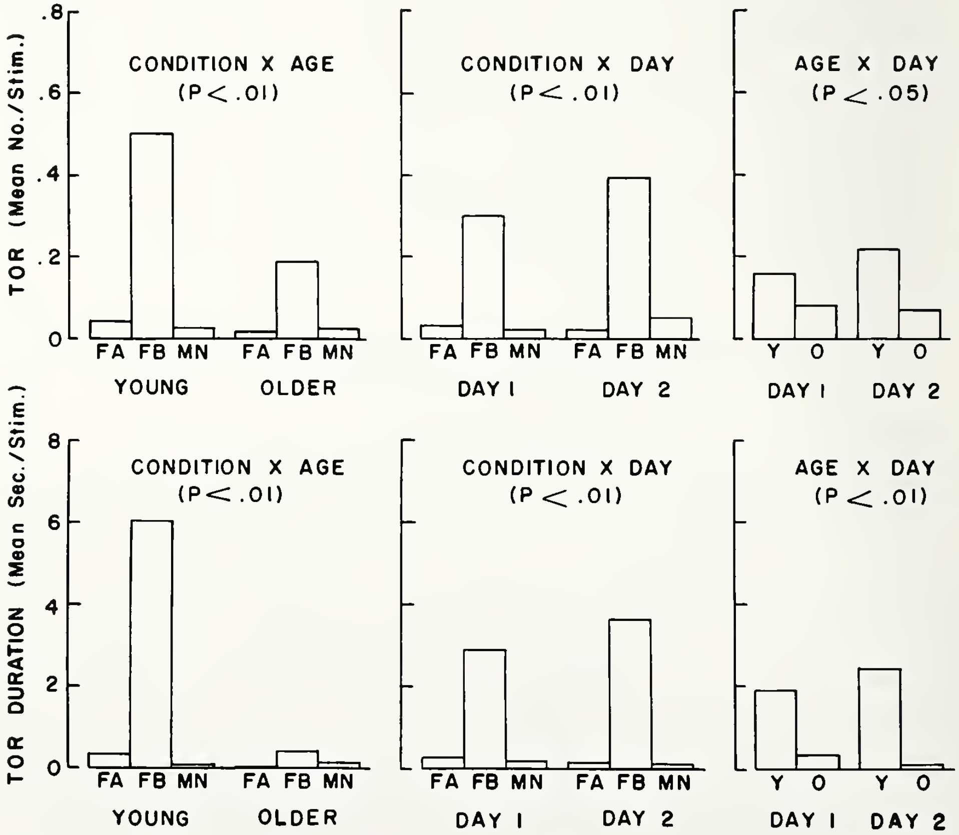

Figure 22–6. TOR (tongue-on-roof of mouth) responses resulting from oral stimulation by finger above tongue (FA), finger below tongue (FB) and milk-containing nipple above tongue (MN) stimulation: other abbreviations are: *Y*, younger and *O*, older. The maximal no./stim. = 1.0 and maximal duration = 120 sec. The probability (*p*)values are based on analyses of variance carried out separately on the number and duration measures.

pies and on Day 2 than on Day 1 of testing (Fig. 22–6). Differences correlated with age were also larger on Day 2 than on Day 1 (Fig. 22–6).

In contrast, the TOF in younger puppies was the most frequent and most enduring response to MN, intermediate to FA, and only occasional and very brief to FB. But the TOF in older subjects was relatively infrequent and relatively brief to both FA and MN and very occasional and very brief to FB (Fig. 22–7). Differences in TOF correlated with conditions of stimulation were greater on Day 2 than on Day 1, but only for TOF duration (Fig. 22–7).

Sex of puppies had statistically reliable effects, but only on TOR duration in one of the two instances of reliable higher order interactions (Fig. 22–8).

It is evident from the data that maladaptive feeding behavior in infant dogs varies with the nature and conditions of repeated oral stimulation as well as with the infant dogs' ages within and between the two test days. Significantly, however, the TOR-TOF states observed

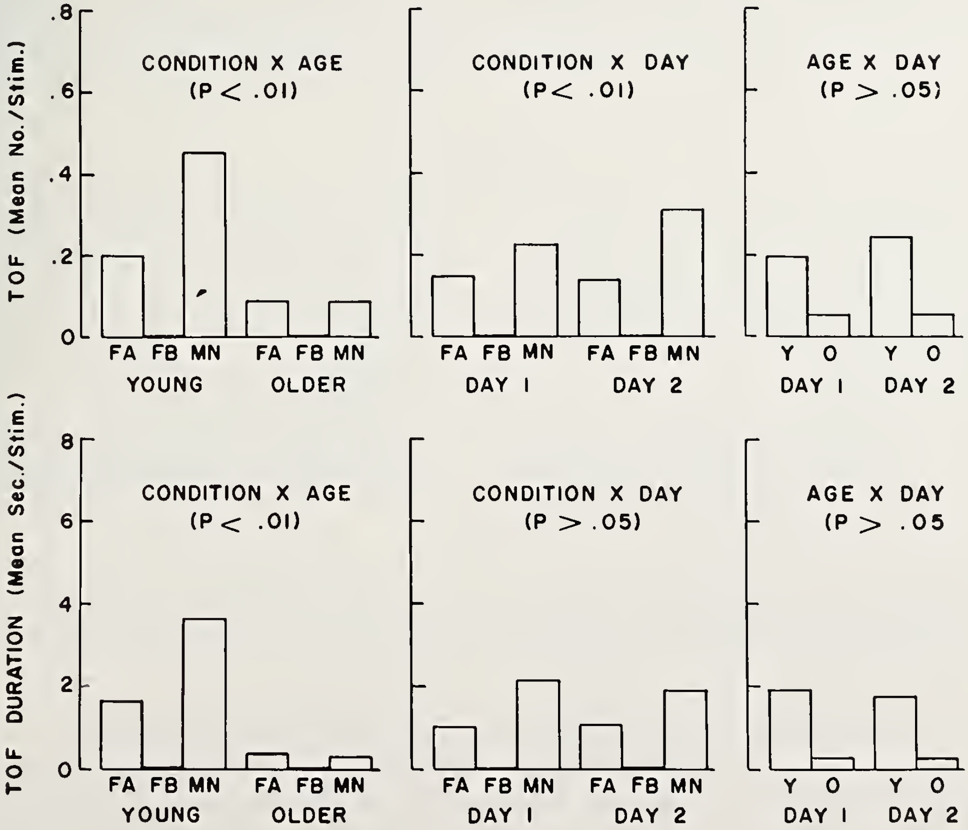

Figure 22–7. TOF (tongue-on-floor of mouth) responses resulting from oral stimulation by finger above tongue (FA), finger below tongue (FB) and milk-containing nipple above tongue (MN) stimulation. Other abbreviations are: *Y*, younger and *O*, older. The maximal no./stim. = 1.0 and maximal duration = 120 sec. The probability (*p*) values are based on analyses of variance carried out separately on the number and duration measures.

in this experiment were not judged extreme or severely abnormal. Indeed they appeared relatively mild when judged by standards of aberrancy set by extreme subjects in the operant key-lifting situation. Many factors might be responsible for this mildness, including absence of specific response requirements, smaller number of stimulations within sessions, and smaller number of sessions, as compared with the operant situation.

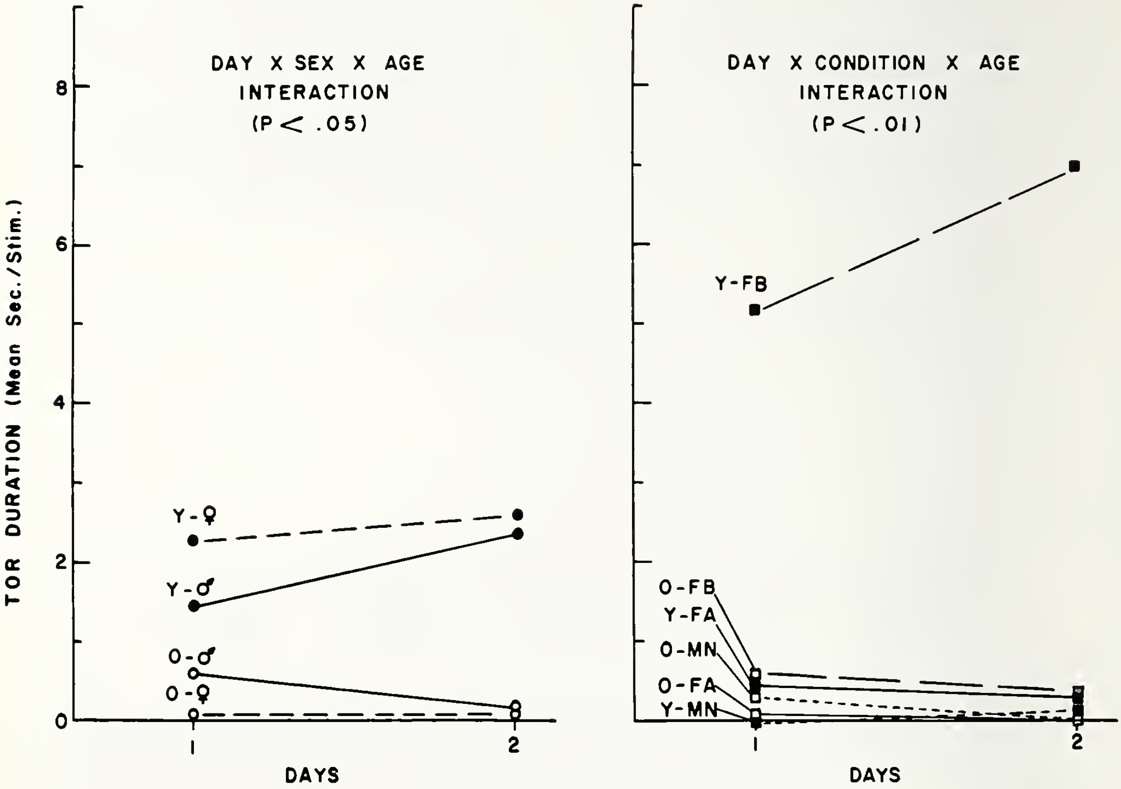

Figure 22–8. Duration of TOR responses broken down to depict the two sources of reliable higher order interactive variation only one of which (left graph) involved sex of subjects.

FUNDAMENTAL NATURE OF THE TOR-TOF SYNDROME

What is the fundamental nature of the motor abnormalities comprising the TOR-TOF syndrome? The behavioral mechanism seems to be as follows: (a) induction of lingual and related dystonias in (b) situations requiring coordinated and directed activity. The consequence of *a* and *b* is (c) a dyskinesia. If both *a* and *b* are present there is the potential for an extensive and remarkable sequence of motor abnormalities which characterize adjunctive TOR-TOF states in infant dogs. If *b*, the ongoing coordinated and directed activity, is absent, then lingual and oral dystonias may occur as they did in the experiment just described, but they lack any aura of pronounced abnormality. Indeed, in normal feeding the tongue of the puppy clearly assumes both TOF and TOR positions transiently. It is the dystonic prolongation of either tongue position instigated during coordinated and directed activity that creates the truly remarkable abnormal behavioral sequences.

The oral abnormalities comprising the maladaptive state invite comparison with "tongue sucking" during the nursing of human infants[11] and drug-induced clinical syndromes or oral dystonias and dyskinesias in human adults.[1,2,3,7,8] Such comparison will require further research, both on the behavioral level as illustrated in this Chapter and on the neurophysiological and neurochemical levels. It may be, however, that the abnormal state reflects, in part, immaturity of subcortex, including basal ganglial and cerebellar functions. It is also concievable that some oral dyskinesias and dystonias might originate during infantile nursing, then be expressed later in ingestive or other situations requiring coordinated oral activity. The profound interference with directed and competent feeding behavior also merits consideration for study in relation to human tics, mannerisms, and posturings as found in mild (neurotic) and severe (psychotic) behavioral disorders.

SUMMARY

The motor abnormalities comprising a newly discovered syndrome of maladaptation in infant dogs include lingual dystonia and stereotyped dyskinesia of postural and related activities. The two distinctive patterns of the syndrome are TOR (tongue-on-roof of mouth) and TOF (tongue-on-floor of mouth). The largely lingual aspects of TOR were first observed in infant beagles in our laboratory some years ago during study of sucking and during maintenance feeding with artificial nipples. Discovery of TOF was more recent and occurred in an operant key lifting situation designed and used for research on learning in neonatal infant beagle dogs. Sequential components of TOR and TOF variants of the syndrome are characterized by tongue position (TOR or TOF), loss of normally directed behavior, stereotypic and repetitive movements, and sudden disappearance of motor abnormalities with concomitant sudden return of lingual and other mobility. One infant beagle Subject spent over one-third of its time in the TOR-TOF state while in the operant apparatus. Another spent only 0.8% of its time in the TOR-TOF state.

The nature of oral stimulation (finger above or below tongue or milk nipple above tongue), age of infant beagles and amount (days) of testing were found to be differentially correlated with lingual components of TOR and TOF.

Psychologically, the maladaptation appears to be a form of intermittent-feeding-schedule-induced adjunctive behavior. Neurologically, lingual dystonia appears to be primary and the dyskinesia secondary. Implications for research and for pathological classes of human behavior are noted.

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DISCUSSION

J.V. BROWN: I have seen this done by some babies in one of my studies.[1] In this experiment, babies had access to a regular nipple for approximately 10 sucks after which the nipple was pulled out and they were presented with a blunt nipple (a nipple from which the elongated portion had been cut). Some infants became extremely frustrated by this procedure and it became nearly impossible to insert even the regular nipple. I have also seen an extreme case of tongue sucking in a chimpanzee such that it was impossible to insert the nipple in the mouth of the animal.

KORNER: Dr. Stanley, have you seen any long term developmental consequences of this abnormality?

STANLEY: So far, we have no evidences of long term consequences.

CAMPBELL: In some of the sucking studies that we have done I have seen a similar response. The withdrawal of an optimal nipple, to put in a non-optimal nipple, led to the tongue going up to the roof of the mouth and the baby tending to become agitated. It was difficult to insert another nipple.

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[1] Brown, J. V. Instrumental control of the sucking response in human newborns. *J. Exp. Child Psych.*, *14*:66–80, 1972.

Chapter 23

THE INFLUENCE OF CONTROLLED ORAL STIMULATION ON TONGUE MOVEMENTS IN PATIENTS WITH ORAL FACIAL DYSKINESIA: ELICITATION OF LATERAL TONGUE MOVEMENTS

Bradley T. Thach and James M. Weiffenbach

INTRODUCTION

The syndrome of involuntary movements of the face and mouth termed oral facial dyskinesia (OFD) is a commonly reported entity which is usually iatrogenic (phenothiazines, L-dopa) and less commonly idiopathic.[1,3,7,11,13,19,21] Detailed description of tongue movements in OFD are uncommon and yet impairment of lingual motor control contributes to abnormalities of mastication, swallowing and speech which are associated with the syndrome.[7] Attention has been drawn to the significance of sensory input in extrapyramidal disorders.[17] Furthermore, both diffuse tactile stimulation and anesthesia are said to inhibit involuntary oral movement.[2] We have developed quantitative methods for oral touch stimulation to study elicited tongue reflexes in normal newborn infants.[24,25,27,28,29] We have taken the opportunity to apply these methods to the study of three patients with oral facial dyskinesia and five adult control subjects. In the following report we shall (1) fully describe the abnormal oral and lingual movements in these three patients, (2) describe the effects of controlled oral sensory stimulation of the abnormal movements and (3) speculate on the possible relation between the elicited lateral tongue reflex seen in normal infants and similar elicited movements in the patients with oral facial dyskinesia.

DESCRIPTION OF PATIENTS

Patient 1: A 65 year old female was referred for evaluation of involuntary facial movements. Three years prior to this she had gradual onset of blepharospasm which was followed within two years by progressive difficulty in keeping her mouth closed. During the course of a conversation, her speech would become

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unintelligible. She drooled and had occasional choking and nasal regurgitation of liquids while swallowing. She became dyspneic when these symptoms were severe. Symptoms were increased by anxiety or fatigue, but were observed to disappear during sleep. Tranquilizing drugs produced no lasting change in her symptoms. There was no family history of degenerative neurological disease, or history of encephalitis or of exposure to phenothiazines. The patient had a complete dental extraction at age 35. She did not wear lower dentures. One year after onset of symptoms a trial with newly fitted full dentures did not improve the dyskinesia.

On examination, intermittent and irregular platsymal contraction, blepharospasm, and yawn-like mouth opening were observed. The frequency of these movements was variable but there was usually at least one abnormal movement every 8 to 10 seconds. The tongue tip alternatively darted to either side, protruded against the lower lip or withdrew backwards into a ball in the center of the mouth. An up and down 3 to 4 per second tremor, which was fairly regular, involved the mandible and tongue. The soft palate moved up and down irregularly with a frequency of about once per second. Speech was labored and barely comprehensible. Speech was accompanied by harsh gutteral sounds presumably of laryngeal origin.

Lingual coordination in following a moving object was fair. Blepharospasm was increased by repeated tapping on the glabella. The patient was restless and frequently paced about the room. The oral sensory examination, including two point discrimination, touch, and taste, was within normal limits. There was a slight terminal tremor of the left upper extremity when pointing. The rest of the neurological examination was negative. Notably absent were rigidity, weakness, wasting, peripheral sensory deficit, evidence of decreased mentation or psychiatric disease.*

Patient 2: A 55 year old female was evaluated for involuntary movements. Five years previously she noted insidious onset of blepharospasm which was followed over the next 6 months by the gradual appearance of involuntary movements of the mouth, tongue and hands. She had difficulty keeping food in her mouth while eating, had choking episodes while swallowing, nasal regurgitation of solid bits of food, and stiffness in the neck. She fidgeted with her hands and developed chronic ulcers of her forearms from constant picking. The involuntary movements increased with anxiety and disappeared during sleep.

Except for minimal dose of fluphenazine (1 mg per day) taken for one week three months prior to onset of symptoms there was no history of exposure to phenothiazines or other tranquilizers prior to onset of symptoms. There was no personal history of encephalitis or family history of hereditary neurological diseases.

On examination, dental occlusion was good. There was a small torus of the hard palate. There were numerous small excoriations of the hands and arms. She had frequent episodes of blepharospasm combined with intermittent irregular tic-like bobbing movements of the head, platysmal contraction, and grimacing. Like the first patient, the frequency of these abnormal movements was variable but was at least 1 per 10 seconds. The tongue tip made frequent licking movements from side to side and in and out across the lower lip. Although verbal articulation was good, interruptions by grimace made speech difficult to understand. The lingual musculature had normal contour and bulk. The tongue was normally coordinated in following a moving object. Oral sensory testing was normal. Tapping on the glabella increased blepharospasm. Aside from these abnormalities, cranial nerves, muscular strength and tone, peripheral sensation and reflexes were all normal. Other than a mild lability of mood, there were no findings indicative of psychiatric disease or intellectual impairment.

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*The results of special studies including serum ceruloplasmin, skull and sinus radiographs, lumbar puncture, brain scan, and slit lamp examination of the cornea were normal in all three patients.

Patient 3: A 73 year old female with the diagnosis of chronic schizophrenia was referred for evaluation of facial movements. She had been hospitalized for over 30 years and had received medication with phenothiazines daily for about 11 years. Six months prior to referral she developed involuntary chewing movements. Unlike patients #1 and #2 she did not appear to be greatly distressed or impaired by these movements. She was edentulous except for four lower incisors. Her mouth was in constant motion with chewing movements and lip pursing. When the mouth was open, the tongue shuttled rhythmically from side to side. The rest of the physical examination was normal. Except for poor memory and psychiatric findings indicative of schizophrenia, neurological examination was otherwise normal.

Control Subjects: Five control subjects who were patients attending the National Institutes of Health Dental Clinic volunteered to be studied. None had a history of neurological disease. Their ages were 34, 38, 49, 57 and 62 years. All were females. One of the subjects (aged 57) was edentulous. One subject, aged 38 years, had a history of Sjogren's syndrome with xerostomia. None had a history of treatment with phenothiazines or neuroleptic drugs.

Examination Procedures

All patients were studied at least once in a standardized testing session which usually was filmed with a 16 mm camera or was video taped. At the beginning of the session, patients and controls were told that they were going to be tested with standard stimulations in order to determine oral sensitivity. The sessions lasted about 20 minutes and consisted of three parts. First, the subject was seated in a comfortable chair and engaged in conversation lasting about five minutes. The patient was asked to relax, open her mouth and "hold still". Then the first of two test procedures, each employing a different kind of stimulation, was begun. The tongue tip was stimulated at 20 second intervals with one of a series of eight nylon filaments of graded stimulus intensity.* There were usually 10 to 16 stimulations in all, equally distributed to right or left side of the tongue. The strongest stimulus in the series was 3.60 grams and the least .06 grams. Stimuli were delivered so that patients were unable to see which side of the tongue was being stimulated or precisely when the stimulation occurred. The tongue was observed for at least 3 seconds after delivery of the stimulus, after which the subject was asked to close her mouth. She was asked to identify the side of stimulation by raising the right or left hand. After lingual stimulation, the lower lip was stimulated one to three times on each side at a paramedian site half way between the beginning of the vermilion border and the line of approximation between upper and lower lips.

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*Semmes-Weinstein aesthesiometers were obtained from the Research Media Co., 163 Eileen Way, Syosset, New York.

During the third part of the session another kind of stimulus was employed. Five μl drops of water at room temperature were applied to the right or left half of the tongue tip using an automatic microliter pipet.* There were 10 to 20 stimulations in all, half to the right and half to the left. This was followed by 4 to 6 water drop stimulations to the lip, half to the right and half to the left. The drop hanging from the tip of the pipet was allowed to flow onto the mucosal surface without contact between the tip of the pipet and the surface. The order of both filament and drop stimulation with respect to side of stimulation was random in all patients.

Patients #1 and #2 were studied in repeated sessions over a 6 month period (Patient #1, 20 studies, 18 of which were taped or filmed: Patient #2, 11 studies, 9 of which were taped or filmed). Patient #3 was studied during 1 session which was videotaped. The five control subjects were studied in an identical manner except that filament stimulations were reduced in number (12 to 16 lingual stimulations and 2 to 4 labial stimulations, in 4 of the control subjects with no labial stimulation in one control subject). Each received 20 tongue and 6 lip water drop stimulations. Studies of the control subjects were videotaped. Notes on tongue and oral movements were taken during sessions and these notes were later reviewed against a replay of the videotape or 16 mm cinema film.

Observations of Tongue and Mouth Movements

In the patients, stereotyped lateral tongue movements occurred against a background of other abnormal oral and lingual movements. There were basically three categories of abnormal oral movements seen in the three patients studied. Chewing or munching movements occurred with the mouth closed. The mandible moved up and down or followed a circular pattern typical of mastication. Movements of this type were seen in Patients #1 and #3. Repeated pursing of the lips was seen in patients #2 and #3. Open-mouthed gaping was typical of patient #1 and occurred to a lesser extent in patient #2.

We observed 3 types of abnormal tongue movements. The first of these was a fairly regular 3–4 per second tremor of the tip and body of tongue. Part of this may have been mandibular tremor transmitted to the tongue; however, when the tongue tip was elevated above the floor of the mouth, the tongue tremor frequently appeared more forceful than mandibular tremor, indicating that the lingual musculature was actively involved. This movement was characteristic of patient #1 and was seen less frequently in patient #2. A second type of movement was protrusion of the tongue. The tongue intermittently pushed against the lower lip for several seconds at a time (Patients #1 and #3), protruded far out of the mouth (Patient #1), or made rapid in and out excursions with the tongue tip appearing between half closed lips (Patient #2).

The foregoing movements are symmetrical with respect to the sagittal plane. The third type of movement was of particular interest to us. This was a lateral movement of the tongue toward the right or left side of the mouth. These occurred in all three patients and were either rhythmic and continuous, or intermittent. In patient #3, when the mouth was open, the tongue went to and fro from one side of the mouth to the other in a rapid wagging motion. This tongue movement slowed down or stopped briefly when the patient was asked to stop moving her tongue but she was unable to inhibit movement for very long.

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*Manufactured by Eppendorf, distributed by Brinkmann Instruments, Westbury, N.Y.

In contrast to these continuous movements, isolated darting movements of the tongue to the side of the mouth occurred intermittently in patients #1 and #2. The total time involved for each movement was less than 1 second. These movements were frequently seen immediately following the enunciation of a word. They did not necessarily occur with equal frequency to right and left. Of 30 such movements observed during one session in one of the patients (#1), 25 were toward the left, and only five were toward the right. The tendency for a preponderance of left-going movements was seen repeatedly in this patient.

Another kind of tongue movement away from the midline was more complex. This consisted of a quick movement to the right or left followed by a movement in the opposite direction crossing the midline and going to the other side and then rapidly returning to midline position. This type of movement was also frequently seen just after a spoken word. It was seen in all three patients. The position of tongue tip during these motions was not the same in the three patients. In patients #2 and #3, the tip of the tongue was out of the mouth, moving against the upper or lower lip. In patient #1 (edentulous) the tongue remained low in the mouth, moving across the mandibular alveolar ridge.

Like the other abnormal facial movements, the lateral tongue movements were involuntary. Patient #1 was aware of the amount of oral activity at any particular time but seemed unaware of specific lateral tongue movements. The second patient was more aware of the lateral tongue movements and attributed them to the desire to wet her lips. However, she admitted that she made movements whether her lips felt wet or dry and was, in fact, unable to prevent her tongue from moving.

Tongue Movements During Oral Stimulation

All intensities of nylon filaments were recognized by the patients at lip and tongue sites. All patients correctly identified the side of stimulation, right or left. Likewise, 5 μl drops of water were almost always felt and the appropriate side indicated correctly.

Tongue stimuli were observed to inhibit certain movements and elicit others. In all three patients, stimuli had the effect of inhibiting movement in progress at the time of stimulation. In patient #1, tremor of tongue and mandible was arrested by filament touch or water drop. This effect was immediate but brief, lasting only 1 to 2 seconds after withdrawal of the probe or release of the drop. In patient #2, tremor was arrested by touch but arrest of movement by drops was less striking. In patient #3, a single stimulus had little effect on the back and forth tongue movements. However, several light touches in rapid succession (about ½ second intervals) or sustained touch (3 seconds) resulted in a tonic deviation of the tongue towards the side stimulated. This occurred even when the patient was specifically asked to avoid moving her tongue toward the stimulus. Touch stimuli or fluid drop stimuli to the lip were usually ineffective in inhibiting lingual movements.

Single stimuli in patients #1 and #2

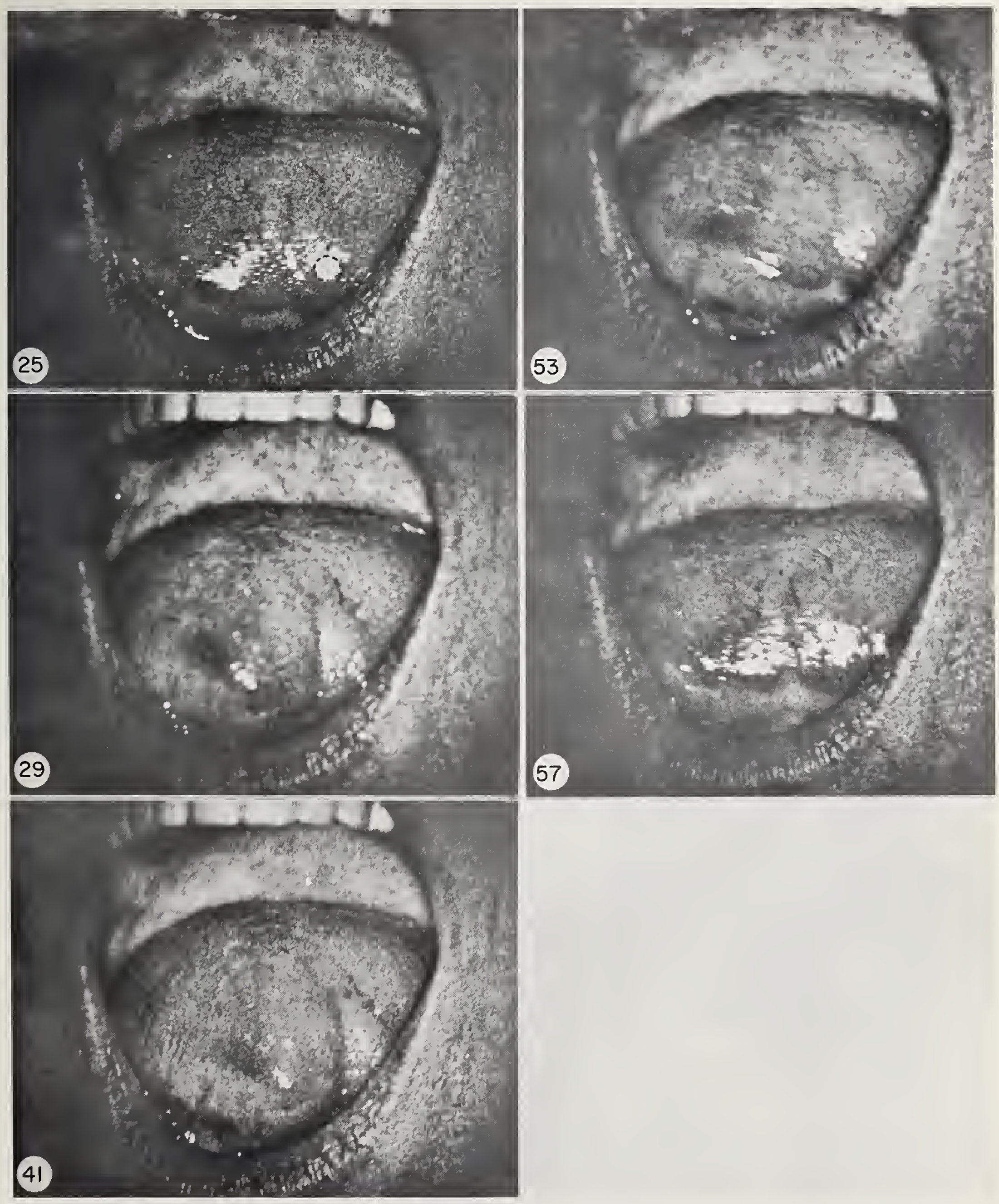

Figure 23–1. Patient #1
Frame prints* showing movement of the tongue to the left following a water drop stimulation to the left side of the tongue. Dot in frame #25 indicates site of stimulation.

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*Film speed was 24 frames per second. Number on frame refers to the serial order of frames beginning with stimulus contact with the tongue surface (frame #1 in each case). The actual stimulation is not shown in each sequence.

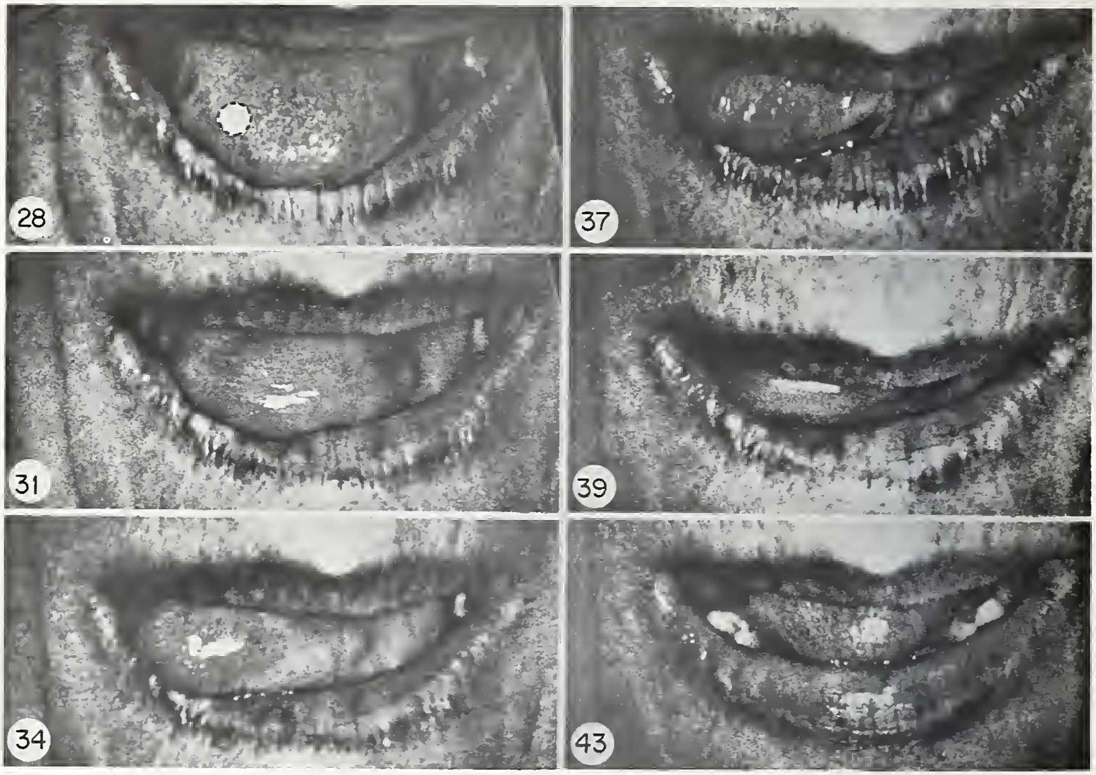

Figure 23–2. Patient #2
Frame prints showing movement of the tongue to the right (#31–37), with the tongue tip moving across the lower lip, following a filament stimulation to the right side of the tongue. Dot in frame 28 indicates site of stimulus.

were apt to result in a rapid stereotyped movement of the tongue tip, usually toward the side stimulated (Table 23–1). The interval between stimulus and response was usually less than 2 seconds but occasionally longer. These lateral movements were identical to the spontaneous lateral movements described above and like them were either single deviations to the side (Fig. 23–1 and Fig. 23–2) or consisted of a rapid side to side movement with at least 1 crossing of the tongue tip over the midline (Fig. 23–3 and Fig. 23–4). Again, right versus left differences were encountered in patient #1: left side stimulation usually resulted in a single deviation of the tongue tip to the left with return to center (Fig. 23–1) and right sided stimulation usually resulted in an initial movement to the right followed by an excursion to the left across the midline before returning to center (Fig. 23–3). In patient #2, this sort of multiphasic movement predominated (Fig. 23–4). Both patients occasionally responded with the initial movement to

Figure 23–3. Patient #1
Frame prints showing a complex lateral tongue movement following a water drop stimulation to the right side of the tongue (frame #1). There is an initial slight movement to the right (frames #47–51) followed by an extensive movement to the left side of mouth (frames #53–60) and return to the center. Arrows indicate the tongue median line.

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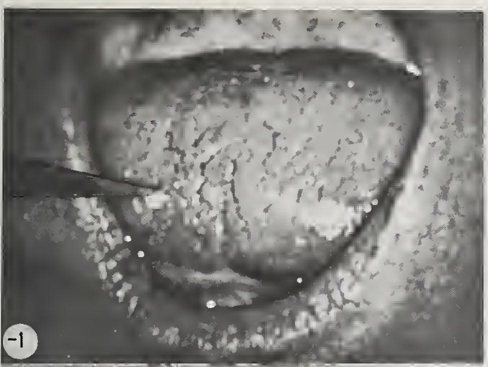
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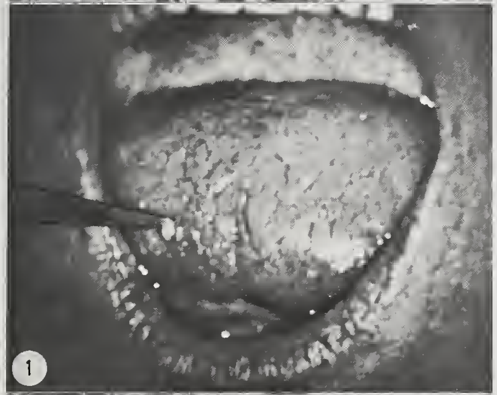
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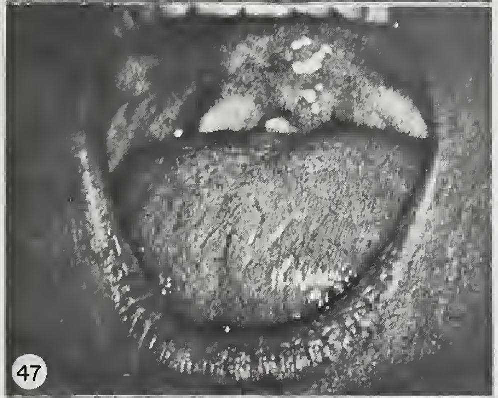
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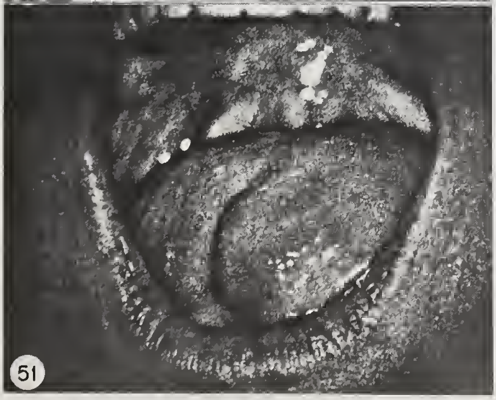
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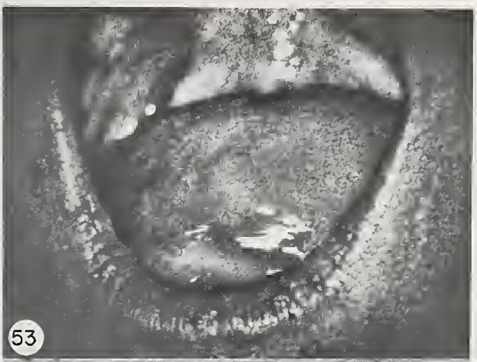
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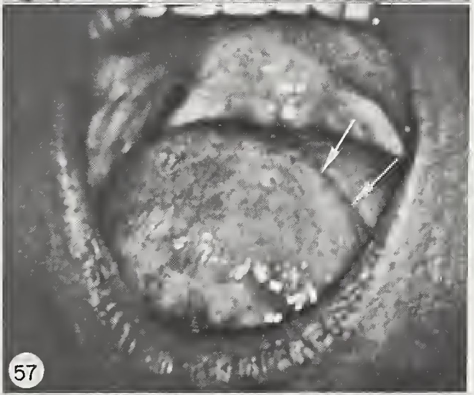
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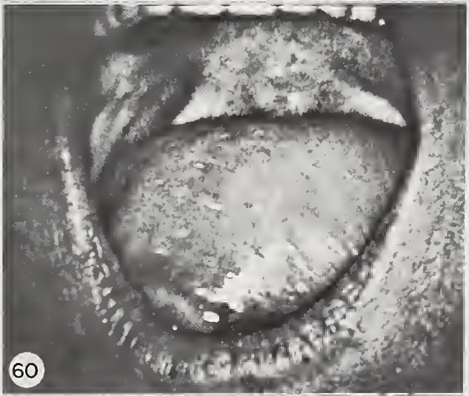
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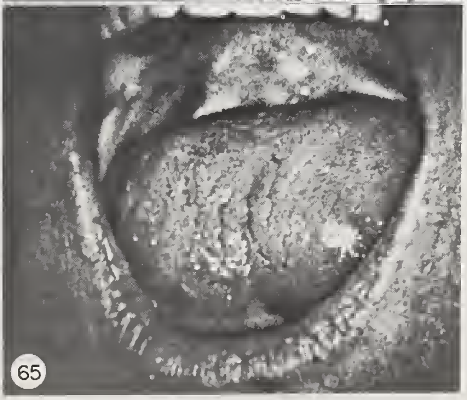
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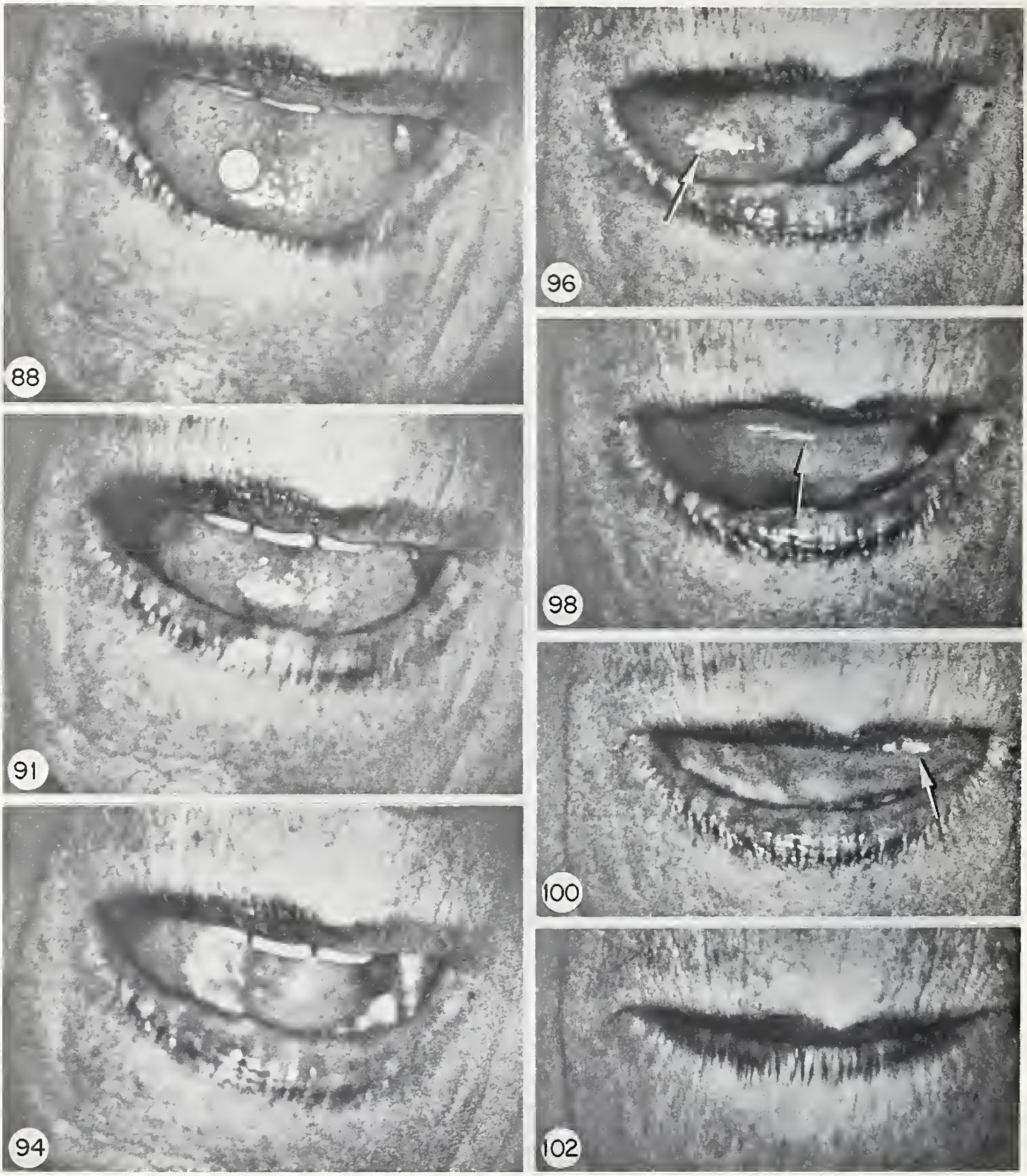

Figure 23–4. Patient #2
Frame prints showing a complex tongue movement following a filament touch stimulation to the right side of the tongue. There is an initial tongue movement to the right (frames #91–96) followed by a movement to the left side of mouth (frames #98–100) as the mouth closes. Dot indicates site of stimulation (frame #88). Arrows point to tongue tip.

the side opposite from the side stimulated but this was infrequent.

The effectiveness of stimuli in producing responses differed. In patient #1 a water drop stimulus frequently produced responses while filament touch was relatively ineffective. In patient #2 filament touch was more effective than water drops in eliciting responses. A stimulus to the lower lip margin instead of the tongue tip resulted in a lateral tongue response occasionally in patient #1 and more frequently in patient #2.

A movement occurring within 2–3 seconds after a stimulation was presumed to be "elicited". That these actually were elicited and not just coincidental "spontaneous" movements is supported by three observations: (1) the initial component of the movement was usually toward the side of the stimulation; (2) stimulation of the tongue and lip were not associated with an equal frequency of response; (3) with patient #1, stimuli of one kind (water drops) were frequently followed by responses but stimuli of another kind (filament touch) were not. If responses following stimuli were purely coincidental, none of these findings would be expected.

Over the six month period that patients #1 and #2 were followed, they both experienced some degree of clinical improvement. Both spontaneous and elicited responses became much less frequent, coincident with improvement in other manifestations of dyskinesia.

Control Subjects

All five subjects were able to maintain a stable oral and tongue posture throughout the session. No tremor was noted. The tongue remained on the floor of the mouth and was inactive. As with the patients, all stimuli were felt and side of stimulus was correctly identified most of the time.

A comparatively slow but definite deviation of the tongue toward the side of stimulation followed water drop stimulations on two occasions (one lip and one tongue stimulation) in one subject. She was a 57 year old female, who had had a total dental extraction one year before and was wearing only her upper dentures at the time of testing.

CONCLUSIONS

These three patients have features typical of the oral facial dyskinesia syndrome which occurs in association with prolonged use of phenothiazines,[8,12,20,21,26,30] use of levodopa in the treatment of Parkinson's disease[3,5] or occasionally "spontaneously".[1] Blepharospasm, chewing movements, lip pursing, mandibular tremor, lingual tremor and in-and-out licking movements of the tongue are reported features of this syndrome.[4,5,12,21,26] Intermittent deviations of the tongue to the side have been mentioned.[13,20,30] In this study, tactile stimulation elicited lateral movements; this has not been previously reported. These elicited movements are strikingly similar to the touch-elicited lateral movements of the tongue in the neonate.[27,28] The chief differences between the patient response and the neonate response are related to the several variations of this movement observed in the patients and the relative frequency of spontaneous lateral movements in the patients. Patients #1 and #2 have "complex" movements in which the tongue crosses the midline, whereas the infants do not. Furthermore, the patients have more frequent spontaneous or non-elicited movements and, when stimulated, are more likely to have a tongue response to the opposite side than the infant. Nevertheless, one is tempted to class the lateral tongue response in these adults with oral facial

FILAMENT STIMULATIONS

	To Tongue			To Lip		
Patient	Number of Stimulations	Responses Ipsilateral	Responses Contralateral	Number of Stimulations	Responses Ipsilateral	Responses Contralateral
No. 1	66	12.1%(8)	4.5%(3)	14	7.1%(1)	0%(0)
No. 2	30	46.7%(14)	10%(3)	13	77%(10)	23%(3)

WATER DROP STIMULATIONS

	To Tongue			To Lip		
Patient	Number of Stimulations	Responses Ipsilateral	Responses Contralateral	Number of Stimulations	Responses Ipsilateral	Responses Contralateral
No. 1	90	63.5%(57)	5.5%(5)	21	28.5%(6)	4.7%(1)
No. 2	33	15.2%(5)	12.1%(4)	15	53.3%(8)	6.7%(1)

Table 23–#1. Lateral tongue responses elicited by filament touch or water drop stimulation to the tongue and lower lip in patients #1 and #2. Ipsilateral and contralateral refer to those responses in which the initial movement was toward the side of stimulation or away from it, respectively. Numbers represent pooled data from 5 test sessions in patient #1 and from 3 test sessions in patient #2. All responses were confirmed by a review of video or cinema film.

dyskinesia along with the so-called "cortical release reflexes"—infantile reflexes such as grasping, groping, suck and palmomental reflexes, which are seen in senile or other patients with cortical damage.[9,14,22] A difficulty with this interpretation is that these "cortical release reflexes" were not present in our patients. Furthermore there is no evidence of cortical disease.

However, if one considers the involuntary movements seen in basal ganglia disease to be release phenomena arising from intact neural mechanisms freed from inhibition by damaged structures within the basal ganglia,[14] then the lateral tongue movements in our patients also are "released" responses. Oral facial dyskinesia is currently speculated to result from increased sensitivity of cells within the corpus striatum resulting from structural damage at the synapse.[6] The involuntary movements of this syndrome are characteristically coordinated movements of associated muscle groups.[12,20] They often appear purposeful in character and may be exaggerations in degree or frequency of normal facial movements. For example, blepharospasm appears to be an exaggeration of the normal blinking reflex. Glabellar tapping elicits blink in the normal individual but this response is inhibited with repeated tapping.[16] In patients #1 and #2, the blink reflex was exaggerated by repeated stimulation and inhibition did not occur. Lateral movement of the tongue in response to an ipsilateral lingual stimulus may not necessarily be abnormal in adults, as its occurrence in one of the control subjects would suggest. It could even be a functional reflex of the adult just as is blinking. In any case, whether it is a latent abnormal response or an unrecognized normal response, like other facial movements, it might be expected to become overt in oral facial dyskinesia.

The relation of the infant lateral tongue response to the adult response is obscure. It is interesting that Demaret[10] has compared phenothiazine induced torticollis to orienting or rooting responses of the infant. A further search for primitive or infantile response patterns, involving the head and neck, in patients with drug induced dyskinesia might prove fruitful.

There are several clinical observations relating sensory phenomena to pathologic oral movements patterns. In describing a disorder characterized by mouthing movements in elderly patients, Appenzeller and Biehl[2] note that a light finger touch to the perioral area inhibits movement. On the other hand, stimulus inhibition of movement in oral facial dyskinesia has not previously been reported. In fact, failure of perioral touch to inhibit movement is regarded as a diagnostic criterion for distinguishing this syndrome from the senile mouthing syndrome.[1]

Proprioception may be an important factor in oral dyskinesia. Since many patients with oral facial dyskinesia are edentulous, it has been suggested that dental malocclusion might be etiologically significant in this syndrome.[15,18] Sutcher et al[23] have described mouthing movements in edentulous patients with poorly fitting dentures which they feel are similar to movements seen in the oral facial dyskinesia syndrome. In four patients, dentures were modified to correct for malocclusion and an immediate and striking improvement in symptoms occurred. They have speculated that the loss of normal proprioception by altered physiologic relationships between mandible and maxilla and loss of sensory input from the periodontal ligament may contribute to dyskinesia. Another observation is that local anaesthesia in patients with senile mouthing reduces these movements or abolishes them for the duration of the anaesthesia effect.[2]

SUMMARY

The three patients with oral facial dyskinesia all had intermittant spontaneous lateral tongue movements. In two patients, stimulation of the side of the tongue or lip with a nylon filament aesthesiometer or with a water drop, elicited predominantly ipsilateral reflex-like tongue movements. The two patients differed with respect to the frequency of response to filament *vs.* drop and lip *vs.* tongue stimulation. The elicited sideways tongue movements in the patients were similar in many respects to touch elicited tongue movements in normal infants. Elicited lateral tongue movements were observed infrequently in one out of five control subjects. In all three patients tongue stimulation had the effect of inhibiting tongue tremor or other tongue movement in progress at the time of stimulation.

Our observations and those reported

elsewhere in the literature indicate that sensory input, either exteroceptive or proprioceptive, can modify abnormal patterns of movement in the oral area. The mechanism of this interaction, however, remains obscure. In the syndrome of senile mouthing, for instance, stimulus input (light touch) or stimulus decrease (local anaesthesia) both have the same effect in reducing movements. Our findings indicate that local stimuli may reduce some types of movement (tremor) or induce other types (lateral tongue response). We have found that both site (lip versus tongue) and type of stimulus (water drop versus touch) may give markedly differing results, which indicates that control of stimulus type and site is important in the study of this phenomenon.

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DISCUSSION

JENKINS: I shall confine my remarks to the abnormal movements of the tongue. These dyskinetic movements of the tongue and mouth are quite common. In adult neurological practice I see an average of one new non-Parkinsonism patient with these per month. Movements of this sort are seen particularly in patients taking levodopa. We now have approximately 250 patients on levodopa for Parkinson's disease and between half and two-thirds of them have, at some time, acquired movements of this general type. These abnormal movements occur in a number of different patterns, even within the clinically homogeneous dopa group of patients. Some of them, like the patient #1 have their lower jaw forced open; this is illustrated by Altrocchi.[1] Other patients have side-to-side movements which resemble the reflex movements demonstrated in infants by Weiffenbach and Thach.[27] Patients may show transverse dystonic bunching up of the tongue with withdrawal of the tip, which you have referred to as "fisting." Patients may also have pointing, guttering, or darting movements of the tip of their tongue which may either hit the palate just behind the upper teeth or pass between the teeth. They may show analogous involvement of facial musculature, with pulling downwards of the lateral parts of the mouth, presumably from contraction of the depressor anguli oris, with or without contraction of the zygomaticus. The platysma muscles are often involved, as well.

We have termed these movements "dystonias". This is appropriate to their patterning, such as the forcing open of the jaw, presumably synergistic with relaxation of the masseter and temporal muscles. The "fisting" of tongue may be dystonic. But the patient #1 seems to have an element of dyssynergia as well. The cinéradographic studies during

[1] Altrocchi, P.H.: Spontaneous oro-facial dyskinesia. *Arch. Neurol.*, 26:506–512, 1972.

swallow showed the tensor palatini muscle contracting when it should relax. And the fiberoptic cinéphotography of the larynx showed synergistic movements of her vocal cords with breathing but dyssynergistic movements with vocalization. Dyssynergia implies that the central template for the patterning of synergies has been impaired. This is a dyspraxia of movements, rather than a simple dystonia.

These abnormal oral motions have been reported in association with a variety of drugs, such as the phenothiazines, the butyrophenones, and possibly the pheniramines (because two of these patients were taking the latter). During the use of the butyrophenones, abnormal oral movements appear within weeks or, in some patients, within days.

Apropos of nomenclature, I believe "tardive dyskinesia" is an unfortunate term. "Oro-facial dyskinesia" is more descriptive, and preferable to "bucco-linqual dyskinesia".

The site and nature of the causative lesion is in question. I don't believe one can envisage the causation of these movements in terms of a specific lesion. There may be a *locus minoris resistentiae*, so that some patients may develop a dyskinesia, whereas other persons with comparable arteriosclerosis or other insults, such as drugs, do not develop them. The cause is probably a cellular abnormality. Though I doubt there is a specific structural lesion underlying the movement disorders, it is possible that stereotaxic surgery may help them. These procedures may be in the fields of Forel, as has been suggested by Dr. Blaine Nashold.[2]

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[2] Nashold, B.S.: The effects of central tegmental lesions on tardive dyskinesia. In Crane, G.E., and Gardner, R., (Eds.): *Psychotropic Drugs and Dysfunction of the Basal Ganglia.* Workshop Series of Pharmacology Section NIMH No. 3, U.S. Government Printing Office, pp. 111–119, 1969.

CONCLUDING COMMENT—I

DAN R. KENSHALO

Dr. Bosma has put together a marvelous array of topics on the development of oral sensation in the fetus and infant. Particular emphasis has been placed on the accumulation of evidence to show that the human neonate has a fully, or almost fully, functional oral sensory system and is capable of discriminatory responses to such stimulation. The topics presented extend from the development of oral sensory and response mechanisms during the first few weeks of life, through birth, to several weeks of postnatal life. There is no doubt that an interdisciplinary approach has been the predominant theme in his thinking when he issued the invitations to participants in this symposium, for I have recognized contributions from biochemistry, dentistry, embryology, histology, medicine, neurophysiology, physiology, and psychology.

We have witnessed experiments conducted on a wide variety of subjects ranging from avians to humans, including chickens, quail, rats, sheep, dogs, rhesus monkeys, chimpanzees, orangutans, and especially human infants.

We have also seen a wide variety of methods employed that involved light and electron microscopy, biochemistry, neurophysiology and behavioral measurement. These extended from the elegantly simple behavioral technique of measuring the rate of ingestion of water, laced with taste substances by Drs. Desor and Maller, to the precise control of all environmental contingencies employed by Dr. Stanley on his puppies in order to study not only exogenous but also endogenous stimuli that alter this feeding behavior during early postnatal life.

It is not possible for me to even touch on all of the important topics that have been discussed in these three days of meetings. It would also be presumptious of me to try to do so for I am woefully ignorant in many of these areas. I cannot hope, for example, to do justice to the importance of the study of oral annomalies, described by Dr. Pruzansky, in the development of oral function.

My comments will be addressed, first, to the important role of the gate or valve at the rostral end of the alimentary canal as demonstrated by the early development of its sensory receptor mechanisms and the presence of apparently innate sensory discriminatory capacities in the newborn.

Second, I shall comment on the important research that deals with the use of the sucking response as a means of getting a "handle" on such important matters as the development of sensory capacity, attending, learning, and social relations in the newborn.

The importance of a functioning buccal discriminatory capacity in the neonate can hardly be doubted. Both Dr. Noden and Dr. Johnston have clearly demonstrated, in their studies of labeled neural crest cells in the chick embryo, that the cells that are to form the sensory and motor systems of the buccal cavity are among the first to migrate peripherally. From these cells are to be developed the Vth and VIIth ganglia, the sympathetic and parasympathetic neurons, and perhaps also the taste receptor cells of the tongue, pharynx, and palate. On the other hand, the degeneration-regeneration studies of taste cells, described by Dr. Zalewski, indicate that taste receptor cells may not necessarily be derived from these migrated neural crest cells. The possible trophic influence of nerves on the differentiation of epithelial cells is an important and much neglected topic of research. But, whether some of the neural crest cells are the primordia of taste cells is not important to the aim of this symposium. The important finding is that these neural structures which innervate the buccal cavity are among the first of the peripheral nervous system primordia to differentiate, not only in the chick but also in the five-week human embryo, as described by Dr. Gasser.

The determiner of the pattern of migration of these neural crest cells is not known. Dr. Johnston suggested that, perhaps, through defective genetic structure, annomalies such as otocephalus and the failure for full development of the maxillary and mandibular bones may be accounted for by the improper migration of these neural crest cells.

Dr. Peach has continued the detailed study of the Vth nerve ganglion by light and electron microscopy in 10–day fetal, natal, and 20–day post-natal mice. Here a striking feature was the presence of light and dark staining neuronal cell bodies that he suggested might reflect the dual embryologic origin of the ganglion cells, some from the placode and the rest from the neural crest cells. When these kinds of structural differences are encountered, one is intrigued by the possibility of functional differences that might be related to them. Unfortunately, none are yet identified.

Dr. Munger described the receptive structures of some of the areas of the newborn rhesus monkey mouth as seen in light and electron micrographs. In the newborn rhesus monkey tongue there appear to be few well-differentiated receptors, except taste buds. Structures, which in the adult have heavily laminated, un-encapsulated accessory structures that invest highly convoluted nerve fiber endings, show only a rudimentary development in the newborn.

In contrast, the tongues of even premature human infants (30–39 weeks gestational ages) are sensitive to tactile stimulation, as described in the observations of Drs. Thach and Weiffenbach. Furthermore, tactile stimulation of the lateral tongue surface elicited stereotyped sideways movements of the tongue tip and body. Longitudinal testing of these infants at two week intervals for the frequency of tongue responses to tactile stimuli gave a negative correlation with postpartum age.

These results demonstrate that the mechanoreceptors of the human infant's tongue function well and one may expect the receptive structures to be well differentiated.

Dr. Thach described for us structures in the inner lips of the newborn that appear to be long, slender, dermal evaginations into the epidermis. A significant proportion of these evaginations contain a prominant vascular loop. While these long vascular loops persist into later life, their prominent appearance in the neonate suggests a functional but, as yet unidentified, uniqueness for this zone of the buccal cavity lining.

Not only do we now have a fair estimate of the sensory structures, but Dr. Hamosh, in an elegant set of experiments, has solved a mystery for us. She has provided concrete evidence of the function of von Ebner's gland which is located close to the root of the tongue. It provides an important digestive enzyme, lipase.

Dr. Kawamura has described for us the reflex mechanisms related to tongue movements in response to lingual stimulation in the adult cats, and Dr. Sumino elaborated on these. Dr. Nowlis presented evidence that the mechanisms necessary for motor control of the tongue are present in the decerebrate cats. Dr. Sumi then described the motor nerve activity in the hypoglossal nerve during swallowing in kittens.

Considered as a whole the evidence from these studies shows that the human newborn possesses the necessary basic sensory and motor equipment for making differential responses to stimuli applied within the mouth cavity.

Two questions now arise. First, if the mechanisms necessary for responses to gustatory stimuli are present, and our earlier speakers have shown that they are, are they functional and can different responses to different taste solutions be demonstrated? Second, if the gustatory system is functional at birth, does it have sufficient sensitivity to act as a discriminator? Or is it like the other sensory systems of the neonate in that only strong stimulation will elicit observable responses?

Most contemporary students of infant behavior contend that the neonate has an extremely limited response repertory. They describe the neonate as exhibiting only different levels of excitement as judged by the amount of motor activity observed. Later, in part through learning and in part through maturation, other emotional expressions including facial expressions differentiated. We now have evidence to the contrary and the answer to the first question concerned with the functional capabilities of the gustatory system. Dr. Steiner has shown us motion pictures of the facial expressions of neonates when strong sweet, sour or bitter solutions were given orally. The facial expressions, lip and tongue movements produced by bitter solutions were convincingly different from those produced by either the sweet or sour solutions. In many instances reliable differences in expression were apparent in response to the sweet and sour solutions.

These different facial expressions, lip and tongue movements appear to be innate. Their innate character is further supported by the fact that they could be elicited in anencephalic and hydroanencephalic as readily as in normal neonates.

The taste solutions used in Dr. Steiner's studies were strong and while he has demonstrated different facial responses to different taste stimuli in the neonate we still face the question of whether this system if sufficiently sensitive to provide a monitor of the neonatal environment.

A partial answer to this question has been described to us by Drs. Weiffenbach and Thach. They showed that three-day old infants make different tongue responses to concentrations of glucose plus water as compared to water alone. Furthermore, Drs. Desor and Maller described a simple preference method in which they measured the volume of water ingested in the 3 min period compared to the volume of water plus a taste substance ingested for a like period following a 2 min rest period.

They found that the ingestion rate of a sugar solution increased as the concentration increased from 0.5 to 3.0 M. Furthermore, the ingestion rates of the neonates varied to equal concentrations of a sugar series (glucose, lactose, fructose and sucrose) in much the same way that adult sensitivity to these sugars varied. On the other hand, they found no differences in the ingestion rates of 0.18 M urea (mildly bitter) or 0.2 M citric acid compared to water. The ingestion rate of salt solutions did not differ from that of water for concentrations up to 0.2 M (strongly salty to human adults). The ingestion rate of the 0.2 M salt concentration was less than that for water, suggesting that that concentration may have had a slightly aversive quality.

The method employed by Drs. Desor and Maller is straightforward and one that has long been used to test performances in sub-human organisms. However, when employed on human neonates a word of caution appears to be in order. These types of investigations should employ the "experimenter blind" procedure for it is well known that nurses impart their own taste preferences to infants in some, as yet unknown, way.

The evidence indicates that the sense of taste, unlike the other senses, is well developed in the neonate. When during fetal development does this innate ability to discriminate taste substances commence? Dr. Robert Bradley has told us that his electrophysiological results indicate that 109 day sheep fetuses (147 day gestation period) possess functional taste buds that respond differentially to several sapid solutions. Dr. Charlotte Mistretta has addressed herself to the problem of determining when during fetal life these taste mechanisms become functionally capable of governing fetal swallowing. We look forward to hearing her results in the near future on the rate of ingestion of amniotic fluid laced with various taste substances by fetal sheep.

One final observation is made. This concerns the use of sucking as a response index. In experiments using sub-human subjects the investigator seeks some response that can easily be made by the organism. He then manipulates the stimulating conditions so as to alter this index of response. Sucking appears to be one of very few coordinated motor activities of which the neonate is capable. Several of the papers presented here during the past few days reinforce the contention that sucking will turn out to be an extremely useful response index to test the sensory capabilities of the neonate. I wish to side-step the issue of nutritive compared to non-nutritive sucking except to suggest that better descriptive terms might be found. Strictly speaking, for example, water or water plus saccharine is non-nutritive and sucking it should be classed as non-nutritive sucking. On the other hand, if we endow the neonate with the intention to ingest food or water it would be called nutritive sucking. Names for these two different sucking behaviors should be clearly devoid of interpretations based on needs, intentions or expectations of the neonate that are assumed by the observer.

What now appears to be badly needed is what the Skinnerian calls "base-line" data on the sucking response. It involves a quantitative description of the response under relatively constant endogenous and exogenous conditions for the neonate. Dr. Stanley has made a good start toward acquiring this basic information by the experimental manipulation of the sucking response in newborn puppies. He has also described some response anomalies induced by intermittant oral stimulation. Dr. Brown has compared patterns of the non-nutritive sucking behavior of several great apes with human neonates 2 to 7 hours postpartum. The great ape neonates appeared to suck continuously at a steady rate punctuated by a few stops, whereas the human neonates showed rhythmic bursts of five to ten sucks, then quiescence for a period of time. The non-nutritive sucking response rate was reduced by strong auditory stimuli, but only a transient rate reduction was produced by visual stimuli. The response appears to be quite powerful and well organized even in human neonates of 32 to 36 weeks gestational age.

In a more detailed study of sucking behavior in early human infancy Dr. Sameroff concluded that neither loud auditory or bright visual stiumli were convincingly effective in altering the sucking rate during bursts. However, as the infant approached 3 months postpartum age, marked changes were produced in sucking rate by both auditory or visual stimuli. Apparently, sensitivity to these stimuli, as indicated by attending behavior, or the mechanism to inhibit sucking does not become functional until then.

While the non-nutritive sucking rate seems to be strong and resists change by external stimuli in human neonates up to 3 months postpartum, Dr. Campbell told us that the total time spent at nutritive sucking can be markedly changed by several environmental stimuli even in the very early neonate. The total sucking time was longer and the rate was higher when infants were held in the arms as compared to lying supine in a crib. This was also true for infants held by multiparae as compared to primaparae. Here then is evidence of a response in early infancy to external stimuli—the mother, who furthers the interaction by learning to manipulate these stimuli.

One of the difficulties inherent in work with newborn human infants has been pinpointed by Dr. Brackbill. When medication is used during delivery, e.g. demerol, the effects on the infant may last well into the neonatal care period. The results of investigation of one and two day postpartum infants may be materially altered by this lingering drug effect.

CONCLUDING COMMENT—II

Samuel Pruzansky

Since this conference is being sponsored by the National Institute of Dental Research, it seemed proper that the final benediction be given by a practicing member of the dental profession who at one time pretended to be a physiologist.

I believe that I speak in behalf of those who have either participated in these conferences or studied their proceedings in expressing appreciation to the Institute for making it possible for Jim Bosma to organize and publish these symposia. They constitute an invaluable reference source that will be studied by students and scholars in a variety of disciplines.

As I reflected on the wide range of topics and expertise in the papers presented, I was reminded of something Paul Weiss[1] wrote:

> The study of life processes is, just as life itself, an unbroken continuum. One may enter it from the lowest end of the scale of complexity, for instance, micro-organisms and single cells, or from the highest end, at which we find ourselves, or at any intermediate point. But, once inside, one must be able to take excursions, conceptually and factually, in any direction without encountering barriers to comprehension and logical consistency. Life science is a single fabric of interconnected facts and concepts in which, as in all dynamic networks, the structure and strength of every portion depend significantly on the condition of all others.

Through these conferences, Jim Bosma helped us discover concepts and methodologies already in use in other fields which were equally applicable to our own. Barriers to understanding that separated diverse disciplines were removed and a substantial common basis of ideas developed between workers in different fields.

Being peripheral to the kinds of research presented at this conference, it would be presumptious for me to discuss individual papers. Instead, I should like to comment on their significance to education in the health sciences.

I am concerned about the inordinately long latent period between the development of important bodies of knowledge such as presented here and their insertion into the curricula of dental and medical schools or into text books of physiology. To accelerate the transference of knowledge from the laboratory to the clinic, would it not be desirable to incorporate many of the procedures described into a format for the physical examination of patients? It would be helpful to have a list of required armanentaria and standardized procedures for evaluating oral sensation and perception in humans of various ages. This would provide a valuable reference for the clinical investigator who is confronted with the occasional but challenging experiment of nature which merits valid and reproducible documentation. If the state of the art warrants such demonstration, might it not be useful to conduct workshops for those interested in clinical studies on oral sensory physiology to assure standardization of methods so that results from various laboratories can be compared?

I am also concerned about the paucity of instruction in sensory oral physiology in schools of dentistry. To illustrate, let me share with you some personal experiences. My first introduction to oral sensory physiology was during my post-doctoral studies at Tufts in 1945–47, when I came in contact with John O'Rourke and Richard Manly. Dr. O'Rourke had co-authored a monograph on oral physiology.[2] I recall that he encouraged me to read widely and outside of my own discipline. At that time, Dick Manly[3] was studying differences in the tactile sensitivity between denture wearers and those with natural dentitions. I was intrigued by the significance of his research and the implications to clinical practice did not escape my attention.

The second push came after I had already decided that I should study the neurophysiology of the stomatognathic system. Warren McCulloch suggested that I read Norbert Weiner's books. There is a passage in one of these books that encapsulated for me an essential concept that had been missing from my instruction as a dental student.

> . . . .The loss of a segment of a limb implies not only the loss of the purely passive support of the missing segment or its value as mechanical extension of the stump, and the loss of the contractile power of its muscles, but implies as well the loss of all cutaneous and kinaesthetic sensations originating in it. The first two losses are what the artificial-limbmaker now tries to replace. The third has so far been beyond his scope. . . . He has no adequate report of their position and motion, and this interferes with his sureness of step on an irregular terrain.[4]

When I read this passage, I realized for the first time how much I, a dentist, had in common with the artificial limb-maker. The best set of dentures I could design would only provide passive support to the face and serve as a mechanical extension of the jaws and the contractile power of their muscles. However, I could do nothing to restore the missing sensory input from the teeth and their periodontal ligaments. I recognized that every time a dentist sought to reconstruct a faulty occlusion through full crown coverage, he contributed to iatrogenic sensory deprivation. While this negative effect should not contraindicate overriding justification for dental reconstruction, the dentist and his patient should be aware of the effect and recognize the need to substitute new sensory cues for those lost.

If I may continue in this personal vein, I should like to tell you about my preparation for an oral examination in physiology in 1953. In the course of my studies, it occurred to me that I could recite at some length on the neurophysiologic control of a variety of bodily functions. However, I was not too sure that I could do the same for the integrated movements of the mandible.

Recourse to standard textbooks of physiology was of little use. (I gratefully recall that it was in Fulton's text that I first found the reference to the demonstration of the monosynaptic reflex in the trigeminal system by Szentágothai). Most texts ignored mandibular movements except for a short paragraph relative to mastication or deglution at the beginning of the chapter on the alimentary system.Nowhere was consideration given to the physiologically coupled aural-oral-facial-pharyngeal-laryngeal-respiratory system that formed the basis for mastication, deglutition, respiration and speech.

Some ten years later when I was invited to participate in a conference on the trigeminal system, I repeated the survey of the available textbooks and found that not much progress had been made to correct these deficiencies. It may be that the paucity of information in our textbooks was a sign that we had not yet developed that critical mass of investigators and scientific literature necessary to have an impact on the educational system.

Since then, there have been some notable changes. Kawamura,[5] for example, published in the Journal of Dental Education an outline of the course work in oral physiology taught at Osaka University. Of course, Dr. Kawamura had a unique advantage. His laboratory did much of the original research that provided the core studies for the curriculum. In addition, as Dean of the Dental School he had the authority, I assume, to foster appropriate emphasis on oral physiology.

There are some equivalent courses in this country, but these are the exceptions to the rule. What I should like to suggest is that it might be useful to sponsor career development awards for teachers in dental and medical science, whose main pre-occupation would be to inventory and assimilate available knowledge into instruction manuals to upgrade the level of education in our schools. This does not imply a moratorium on further research, but rather an emphasis on applying the accumulated products of research into the educational process. I believe we already possess a critical mass of knowledge that can do much to improve the quality of education in all of the health sciences.

The real reason Dr. Bosma invited me was that I might say something about craniofacial anomalies and reiterate the value of such experiments of nature in the study of oral physiology. Such studies have been few in number because of other priorities, because of the limited number of skilled investigators and lack of familiarity with available methodologies.

In the time available, I shall try to titillate your intellectual curiosity by the presentation of but a few anomalies of congenital and acquired origin. First, consider the complete bilateral cleft lip and palate (Figure 1). Do the functions of suckle and swallow in these infants differ significantly from the norm? If these infants are at a disadvantage, what remedies can be suggested? What is the effect of a prosthetic obturator?

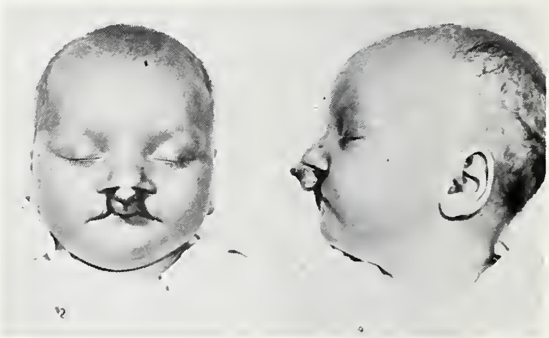

Figure 1. Infant with unoperated complete bilateral cleft lip and palate.

The sensory innervation of the median segment consisting of prolabium and premaxilla is not altogether clear. What happens to the innervation following lip repair? Even after repair, the premaxillary segment containing the maxillary incisor teeth may be mobile (Figure 2). To my knowledge no one has compared the tactile sensitivity of maxillary incisor teeth in a mobile premaxilla with the sensitivity of similar teeth in the non-cleft subject. In situations where fixation of the premaxilla is con-

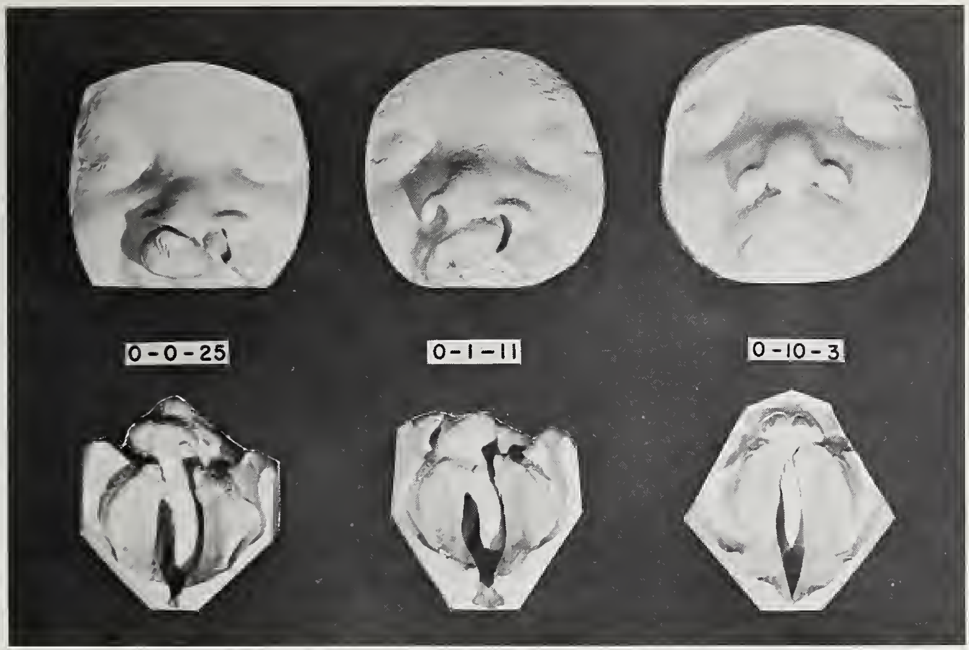

Figure 2. Complete bilateral cleft lip and palate illustrating repair of the lip in two stages. Left, unoperated infant at 25 days of age; middle, at age 1 month and 11 days following repair of lip on right side; right, at 10 months and 3 days following repair of lip on left side.

templated by means of bone grafts, it could be possible to test sensory discrimination before and after surgery.

Let me turn to another experiment of nature, a syndrome known by many names including hemifacial microsomia, first and second branchial arch syndrome, etc. The principal features include malformation of the ear, temporal bone, mandible, muscles of mastication and facial expression on the affected side (figure 3,4). The facial nerve

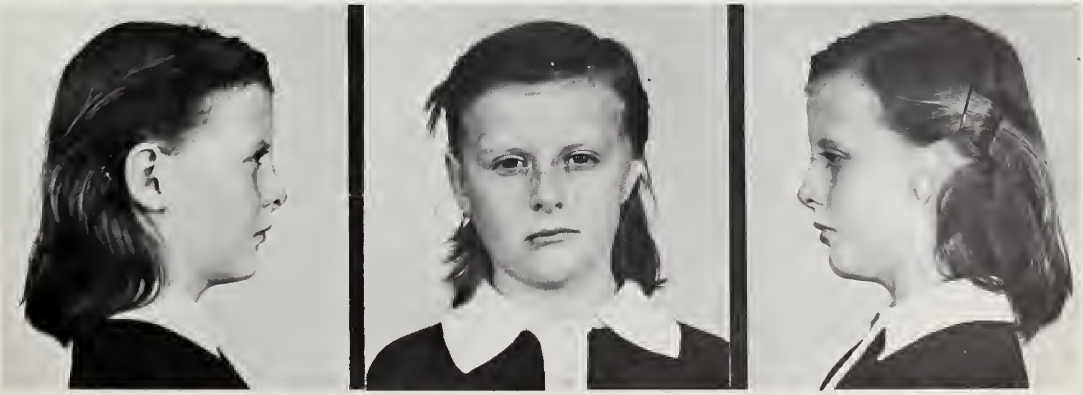

Figure 3. Patient with hemifacial microsomia, age 9 years and 3 months. Note left microtia, asymmetry of lower face, buccal soft tissue and angle of mouth.

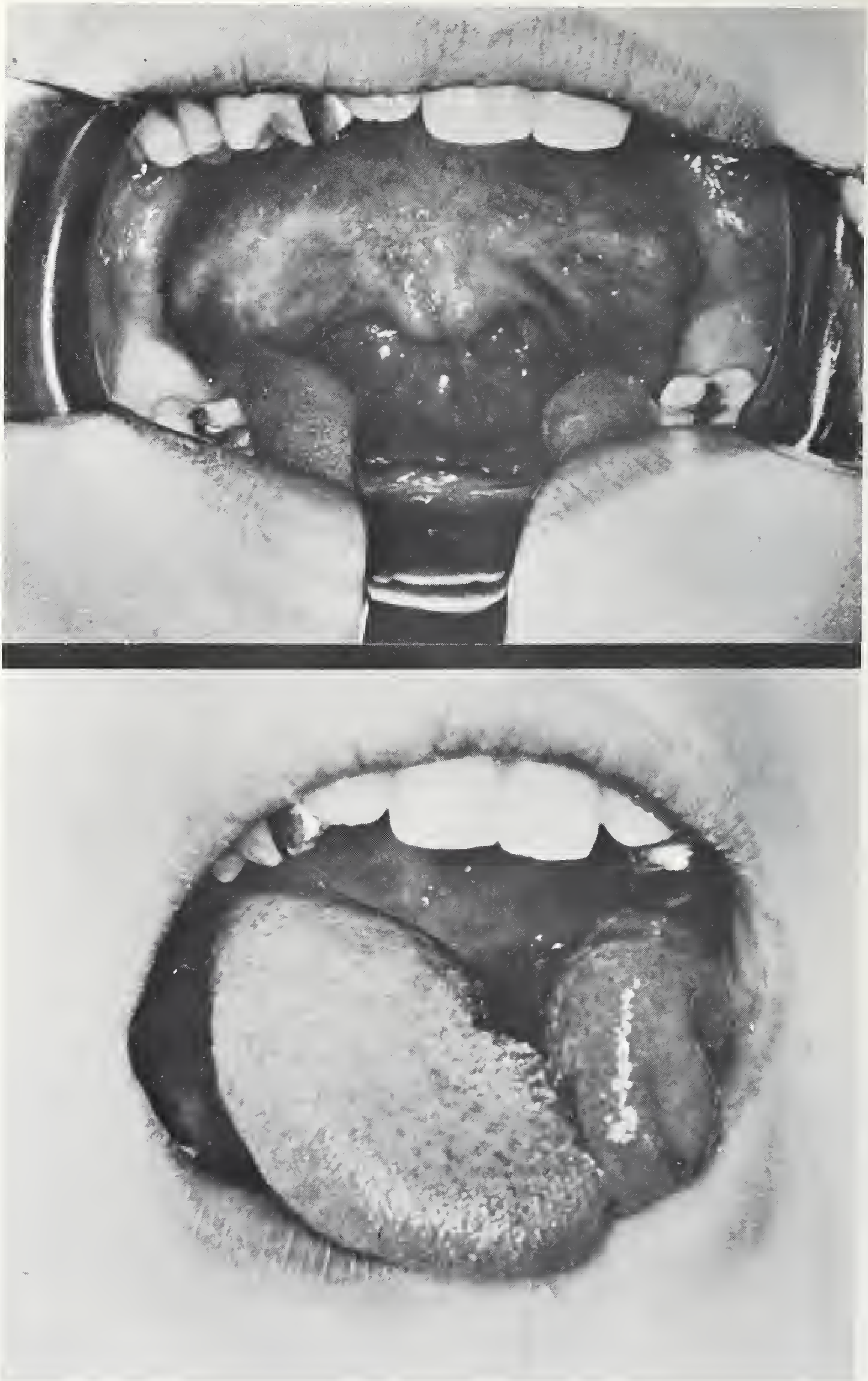

Figure 4. Intraoral views of patient in figure 3. Note bifid uvula and left sided malformation of tongue.

my be affected (Figure 5). The parotid duct, ipsilateral to the microtic ear, may be absent. The uvula and soft palate are often deviated toward the side of the normal ear at rest and during elevation. The eye, maxilla and neurocranium may also be affected. The possibility that the chorda tympani nerve may be anomalous with resultant asymmetry in gustatory innervation remains to be explored.

Many of these patients are now undergoing extensive reconstruction by means of plastic surgery with consequent restoration of oral and facial symmetry. The effect of such changes in oral architecture on oral sensation and perception warrants investigation.

Perhaps the most exciting opportunities for investigation by the oral physiologist are presented by the patient with craniofacial synostosis, such as the syndromes of Crouzon and Apert. Characteristically, the facial deformity grows worse in time due to the asynchronous growth of the midface and neurocranium (Figures 6–9).

The nasopharyngeal airway is often obstructed because of the basilar kyphosis coupled with lack of downward and forward growth of the maxilla in relation to skull base. This forces the patient to become an obligatory mouthbreather. Normal tongue posture is further impeded by the maxillary hypo-

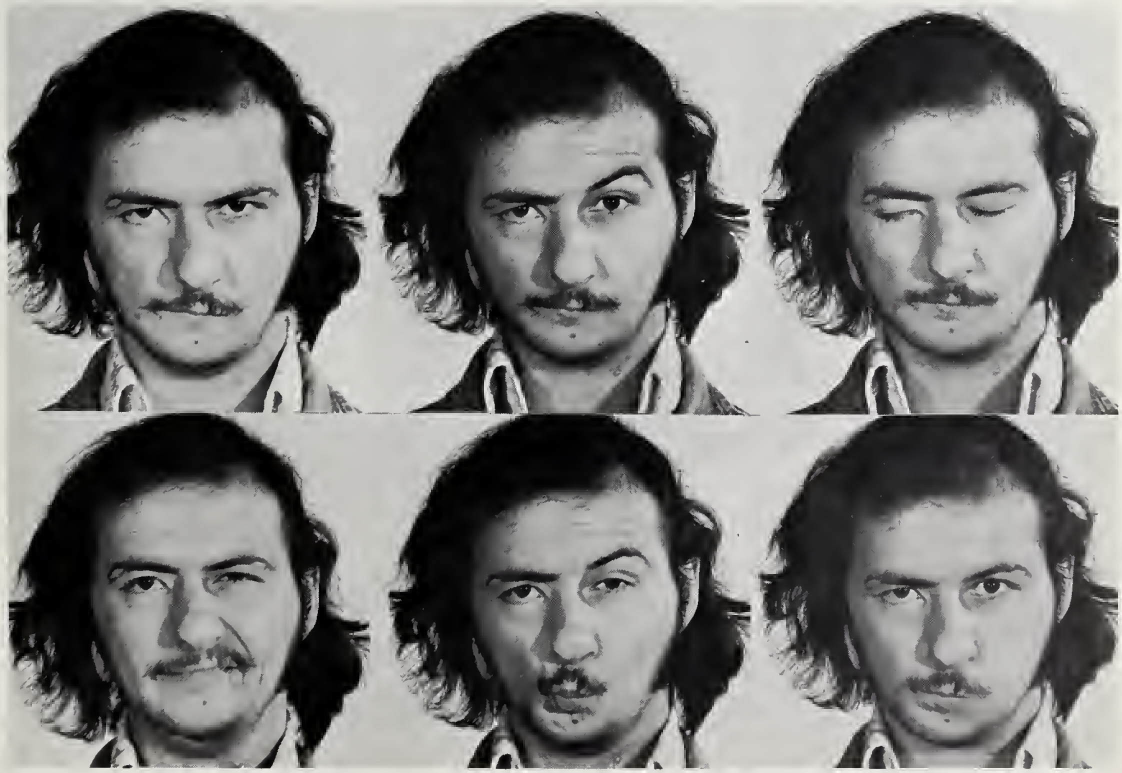

Figure 5. Twenty-two year old male with right hemifacial microsomia illustrating ipsilateral facial nerve weakness. Upper left photo taken in repose; center illustrates asymmetric elevation of eyebrow and furrowing of forehead; right, with eyelids closed gently; lower left, smiling; center, puckering of lips; and lower right, inflating cheeks.

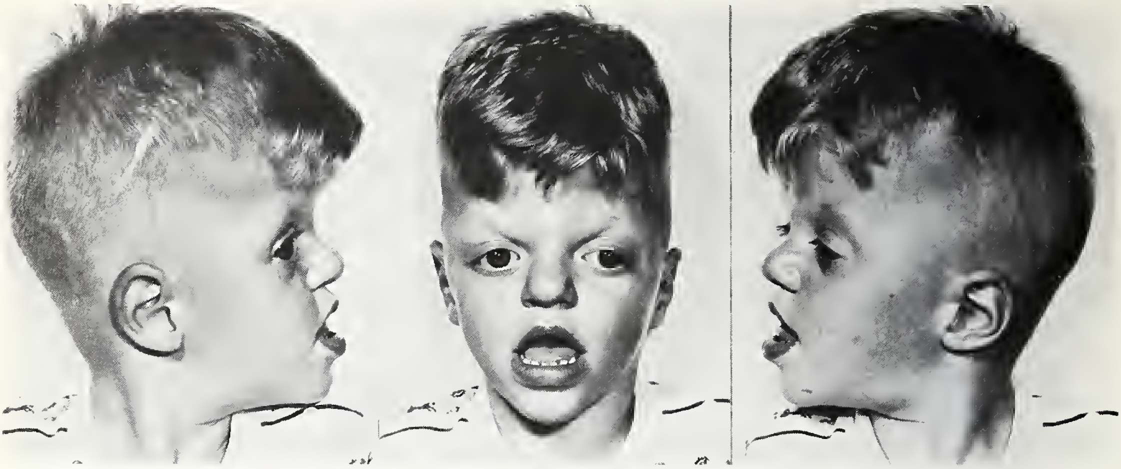

Figure 6. Case #1949, at age 3 years and 10 months. Apert syndrome, acrocephalosyndactyly. Note open mouth posture, protruding tongue, hypoplastic maxilla, hypertelorism.

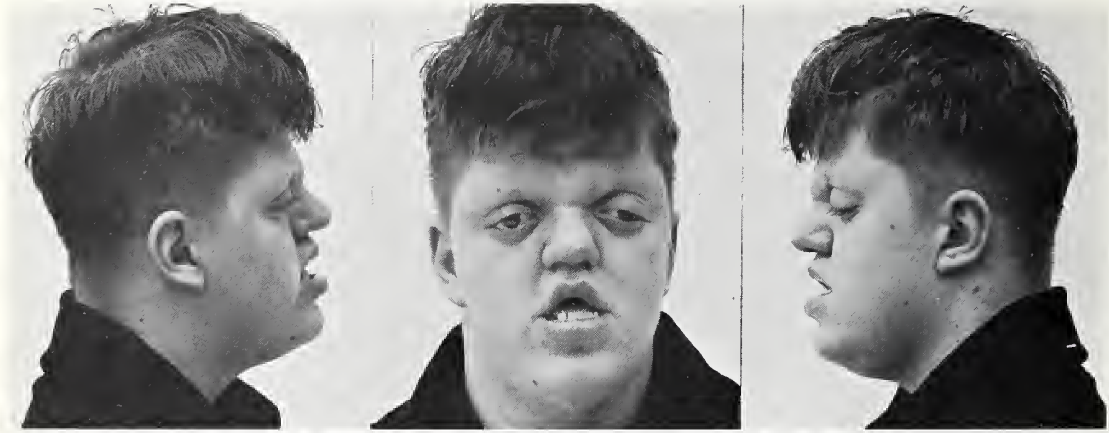

Figure 7. Case #1949, at age 15 years, 4 months.

plasia and peculiar palatal swellings that greatly reduce the size of the palatal vault. The older the patient becomes, the more severe the deformity.

Recent advances in plastic surgery have produced remarkable reconstructions of midface in patients with craniofacial synostosis by detaching the bones of the face from the skull and realigning them with the use of autogenous bone grafts. The extraordinary reconstruction of facial contours and oral architecture must surely induce equivalent changes in function. Studies in oral sensation and perception are currently under way to test the effect of this surgery.

Clinical research appears to be disadvantaged by comparison with research on experimental animals insofar as patients are not all alike, nor is the state of their disease always equivalent. Nevertheless, given a sufficiently large series of cases to form a spectrum of variation, it becomes possible to gain useful

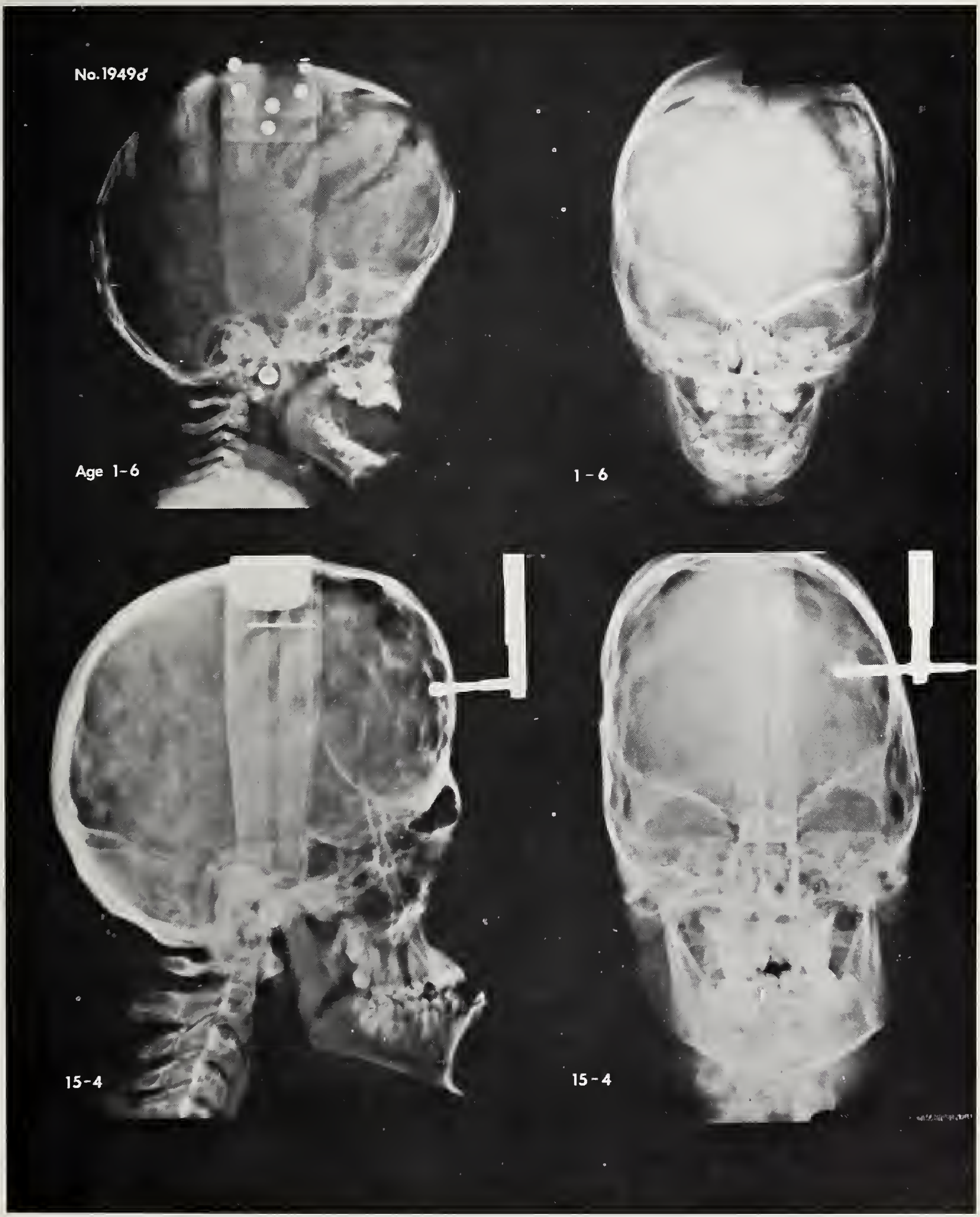

Figure 8. Case #1949. Serial cephalometric radiographs illustrating change from 1 year, 6 months to 15 years, 4 months. Increasing severity of facial deformity due to maxillary hypoplasia. Note occlusion of nasopharyngeal airway, among other radiographic features characteristic for the syndrome.

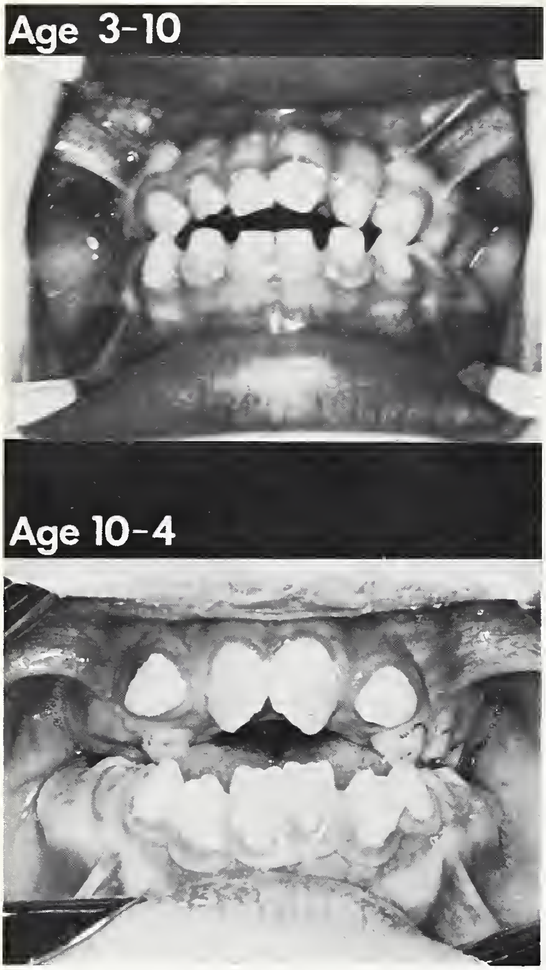

Figure 9. Case #1949. Illustrating increasing severity of anterior open bite.

information. It is in this connection that I want to discuss the defense of the airway.

There is a large series of malformations, particularly those with micrognathia, that threaten the patency of the airway. It is readily apparent that a small mandible can affect the posture and movements of the retromandibular structures and indeed, the postural interactions of skull, mandible, cervical column, posterior and lateral cervical musculature and the supra-and infrahyoid chain of muscles.

Among the various syndromes with micrognathia, such as hemifacial microsomia, mandibulofacial dysostosis (Figure 10), and Pierre Robin (Figure 11), the latter is least able to compensate for the glossoptosis. It is as if the structural defect was also accompanied by some deficit in sensory input from tongue, velum and pharynx that controls tongue posture—as suggested by Bosma.

There is another condition in which the micrognathia develops slowly as a consequence of growth arrest of the mandibular condyles. This may occur following infection or trauma. Where serial records are available, beginning with the early onset of the disease, it becomes possible to monitor both the developing deformity and the functional accommodation. In Still's diesase, a form of juvenile chronic polyarthritis, the development of the mandibular deformity is slow and insidious. The earlier the onset and duration of the disease, then the greater the deformity to be expected. Not only is the mandible smaller, it is also deformed as manifest by the antegonial notching with downward and backward rotation of the chin (Figures 12,13).

How does the patient compensate for the backward and downward rotation of the mandible and consequent distal displacement of tongue and hyoid (Figure 14)? Superimposition of the tracings on foramen magnum, revealed that through compensatory extension of head on the neck (dorsiflexion), the distance between hyoid bone and cervical column was maintained at a constant distance (Figure 15). A similar mechanism was also observed among patients with mandibulofacial dysostosis.

One other case from our series merits illustration with reference to the mechanisms that "defend" the airway.

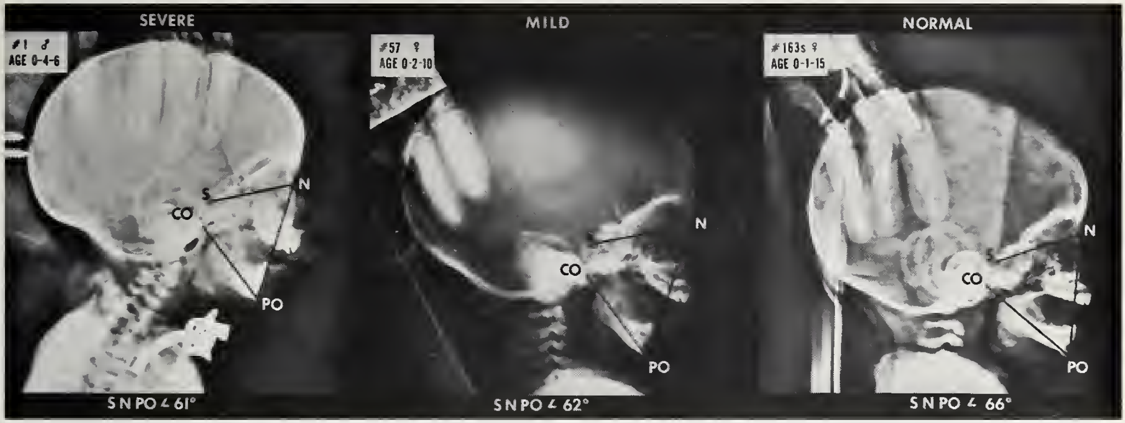

Figure 10. Comparative size of airway, as revealed in lateral cephalometric radiographs, demonstrated in a severe case of Pierre Robin syndrome requiring tracheostomy; a mild case of Pierre Robin syndrome with relatively adequate airway despite the micrognathia; and for comparison, a normal child.

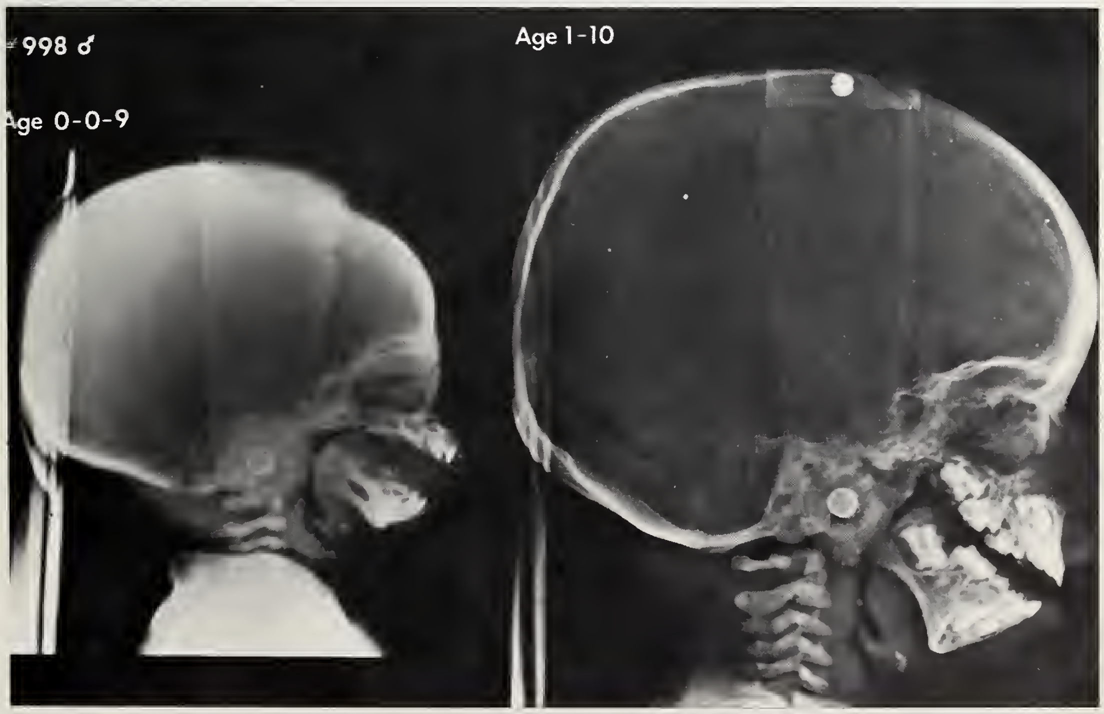

Figure 11. Serial films of a patient with mandibulofacial dysostosis, at age 9 days and at age 1 year and 10 months. Note relatively constricted airway. There was no respiratory distress or gross feeding difficulty.

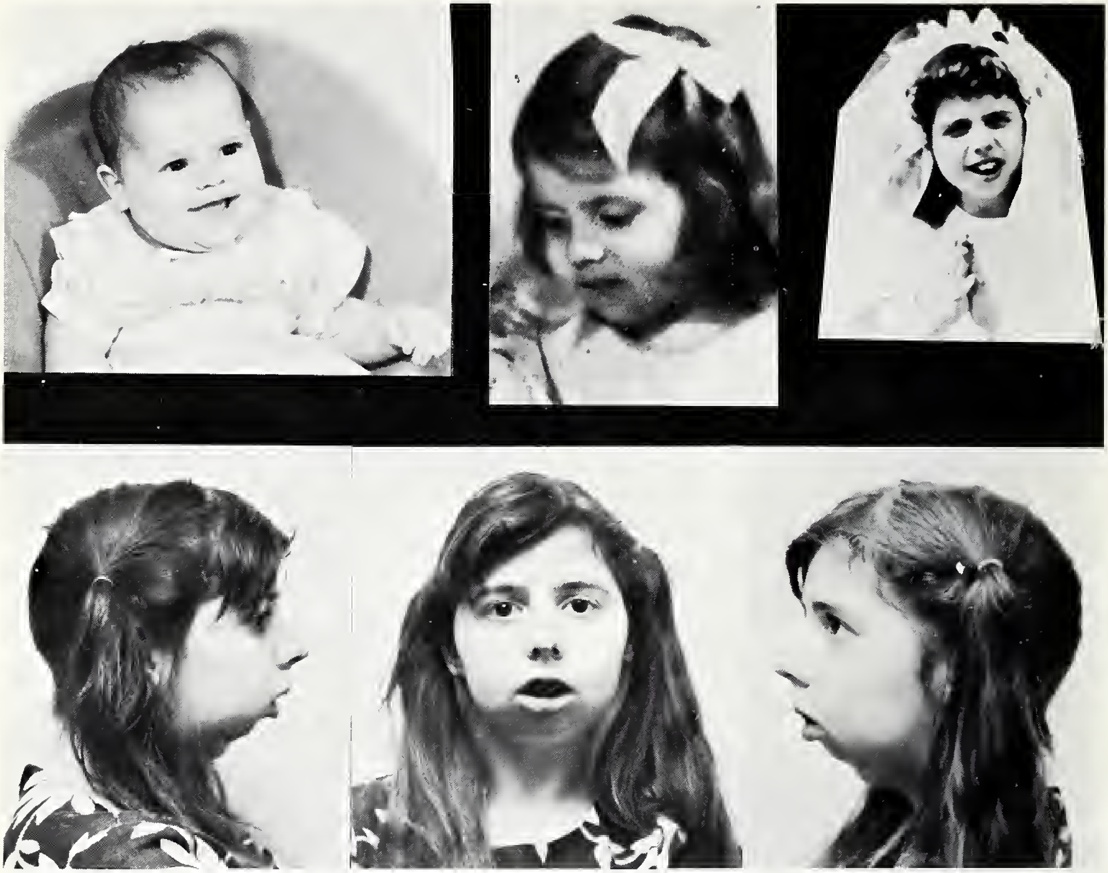

Figure 12. Juvenile polyarthritis (Still's disease) with involvement of the temporomandibular joints. Serial photographs from infancy to age 19 revealed increasing severity of lower facial deformity.

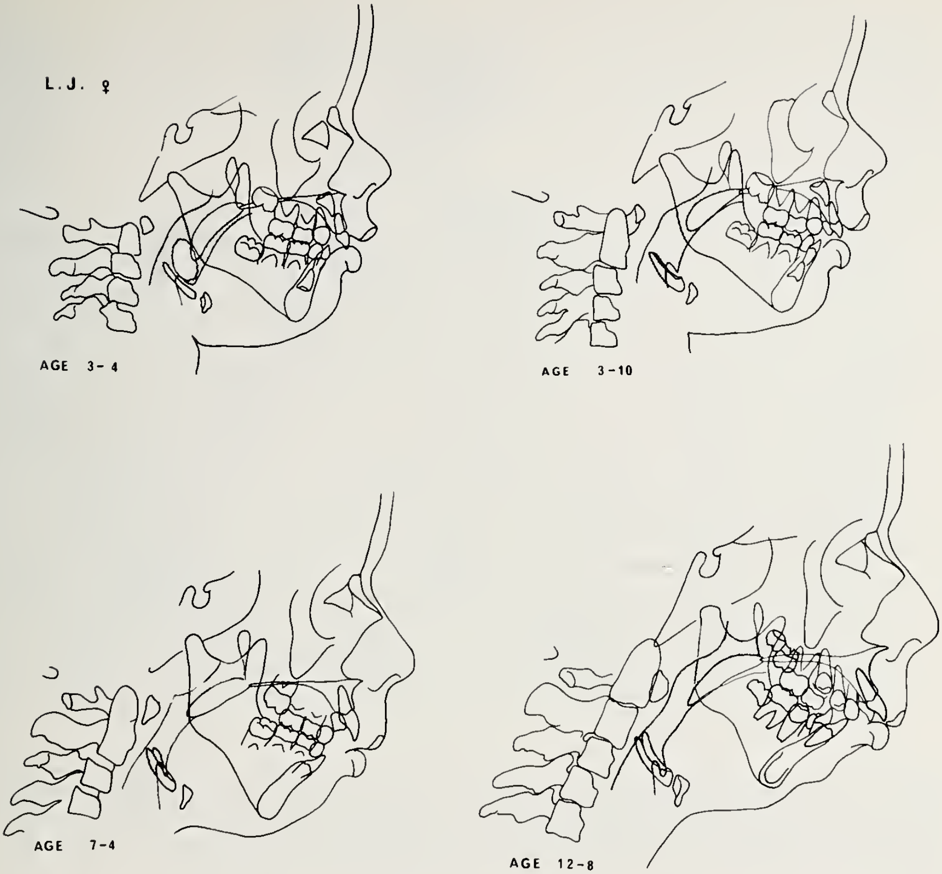

Figure 13. Serial tracings of lateral cephalometric radiographs of patient in figure 12, from age 3 years and 4 months to 12 years and 8 months demonstrate developmental arrest in mandible.

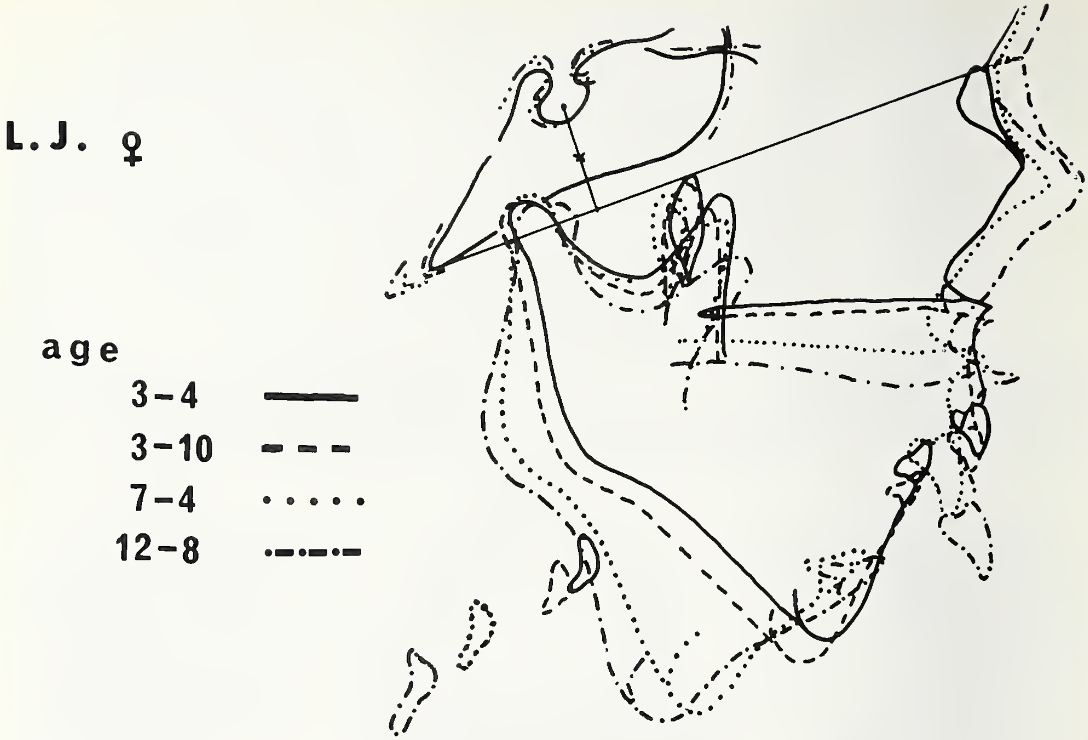

Figure 14. Serial tracings taken from figure 13 and superimposed on skull base to illustrate downward and backward rotation of mandible with similar displacement of hyoid bone. This aberrant pattern of growth accounted for the increasing facial disfigurement and demonstrated the increasing jeopardy to the airway.

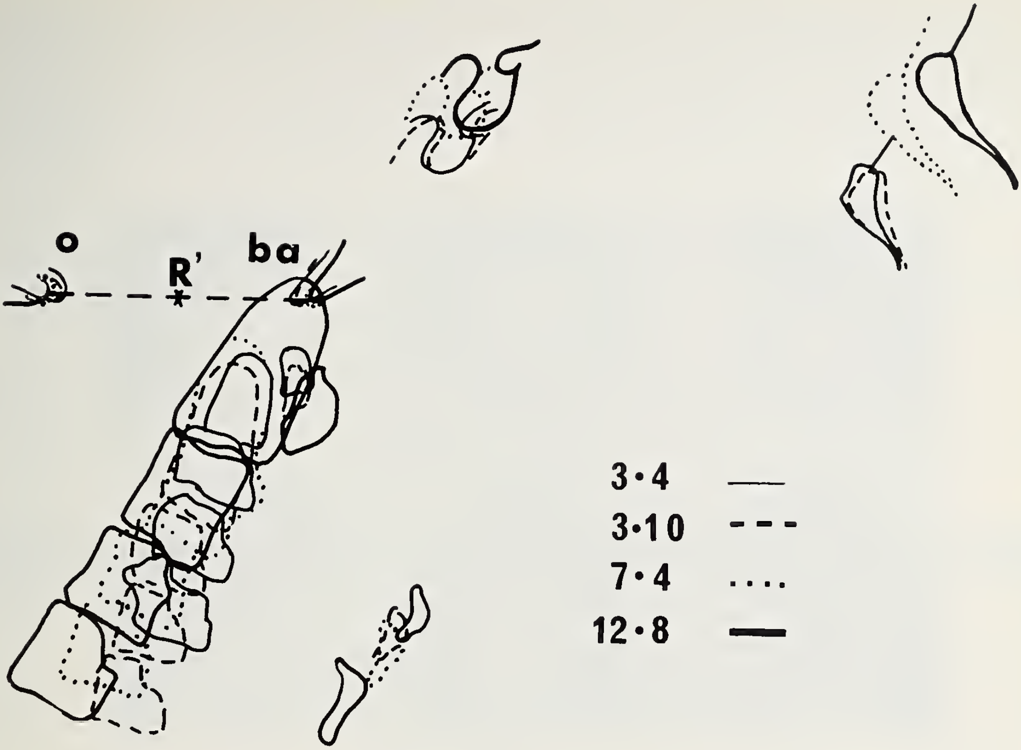

Figure 15. Superimposition of the same series of tracings illustrated in figures 13–14, with registration on foramen magnum. Note increasing distal inclination of cervical column in relation to skull base resulting in maintenance of a constant distance between cervical column and hyoid bone.

The patient presented an arrest of growth of the mandibular condyle on the right side probably due to infection arising in the middle ear (Figures 16,17). Electromyographic tracings of the infrahyoid muscles revealed hyperactivity at rest pre-operatively and gross reduction in electrical activity following

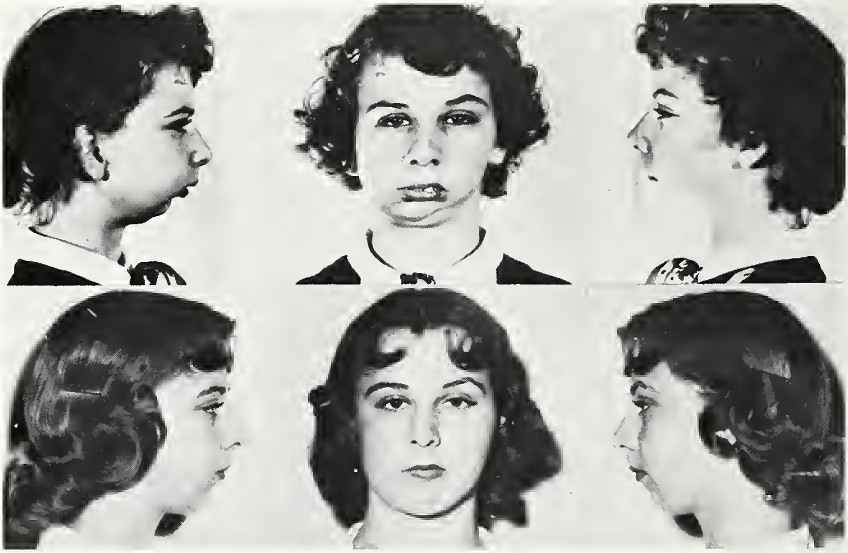

Figure 16. Patient with arrest of growth in the right temporomandibular joint due to infection. Top, before surgery; bottom, following surgical reconstruction.

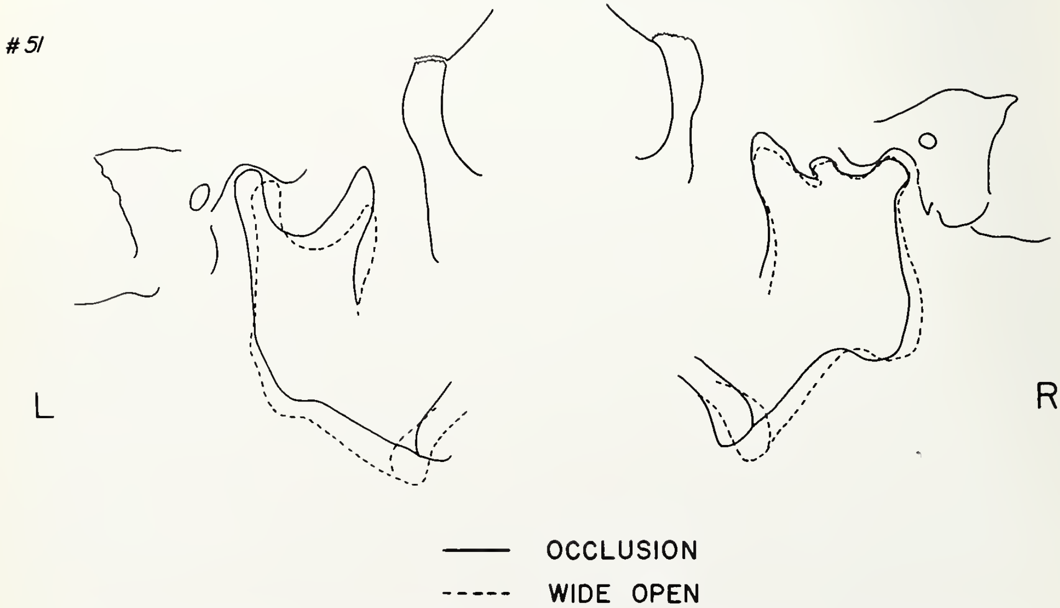

Figure 17. Tracings of pre-operative tomograms of patient in figure 16. Note condylar deformity and antegonial notching on right side.

surgery (figure 18). Serial tracings of the cephalometric radiographs provided an explanation for the electromyographic findings. Following surgery, the airway was widened, the chin realigned in a forward position and the hyoid bone brought upward and forward relative to its former position (figure 19). The radiographic findings suggested that the hyperactivity of the infrahyoid muscles, observed electromyographically prior to surgery, constituted a protective reflex posture mechanism to maintain downward traction of the hyoid and tongue to protect the airway in the presence of a retrognathic jaw.

The mechanism by which antegonial notching is produced in such cases has

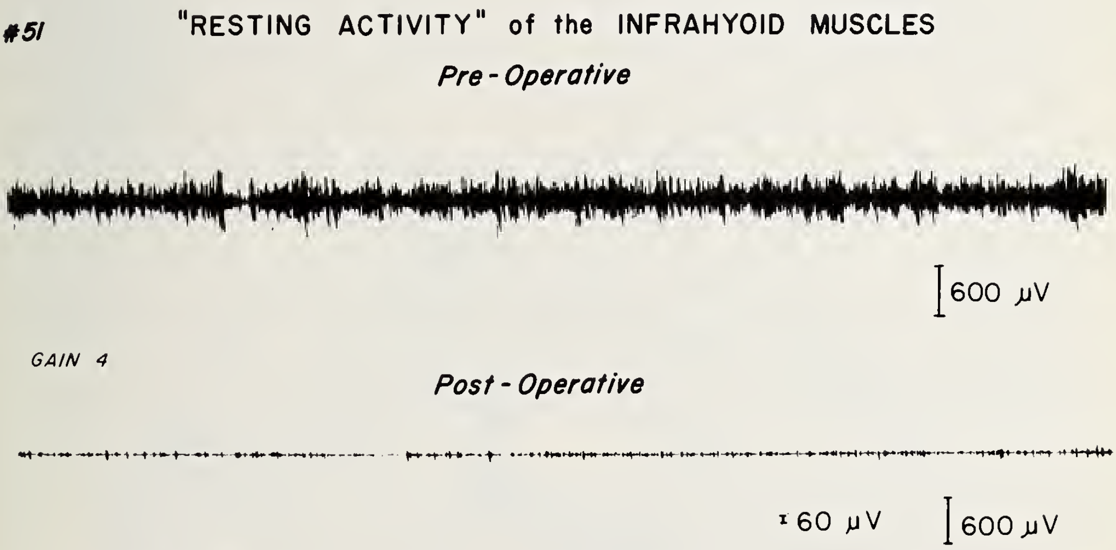

Figure 18. Electromyographic activity of the infrahyoid muscles taken pre and post-operatively in the patient illustrated in figure 16. Surface electrodes detected unusual activity at rest in the pre-operative state which diminished post-operatively.

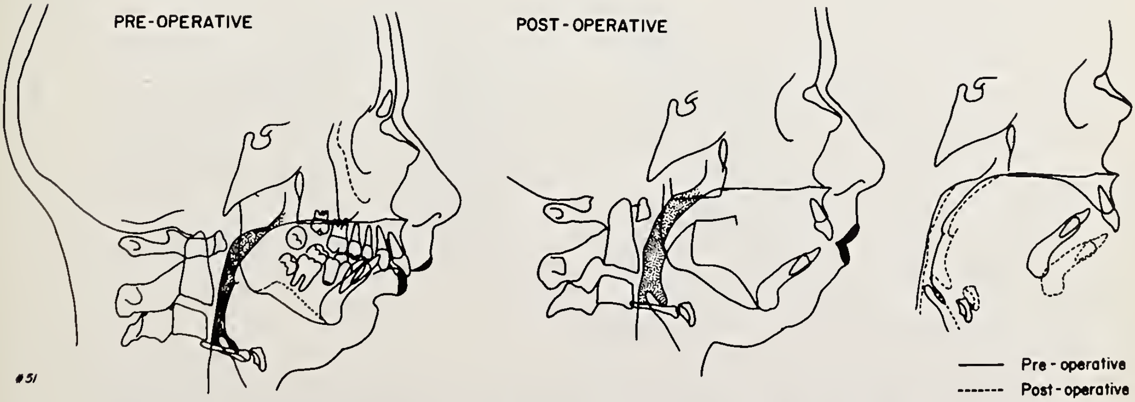

Figure 19. Serial tracings of cephalometric radiographs taken pre- and postoperatively of patient in figure 16. Superimposed tracings revealed alterations in airway, chin and hyoid bone position.

always intrigued us. On the basis of our serial radiographic and electromyographic studies, as well as other clinical observations, we can propose the following explanation: The process of normal growth requires coordinated increments in contiguous structures such as cervical column, mandible, tongue, muscles of

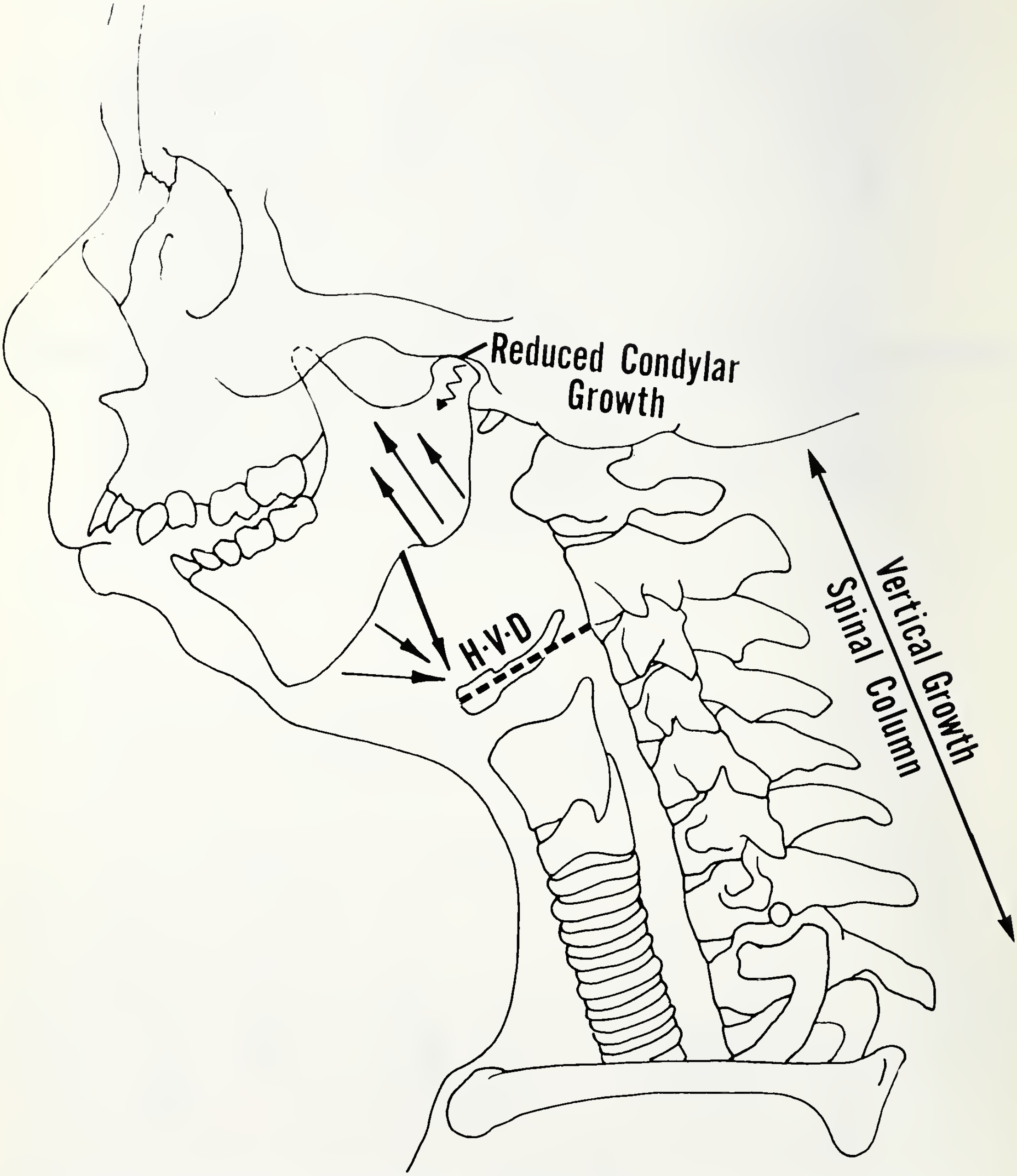

Figure 20. Explanation of antegonial notching due to imbalance between vertical growth in mandibular condyle in the presence of antagonistic muscle forces with the antegonion as the fulcrum. H-V-D denotes the critical hyoid-vertebral-distance that must be maintained to safeguard the airway.

mastication and so forth. Now, if the contribution of the mandibular condyle to this process is blocked, then the system will become distorted by virtue of continued growth elsewhere. This is manifest in the distorted antegonial notching at the interphase between the elevators and depressors of the mandible (Figures 21,22).

From this small sampling of a large

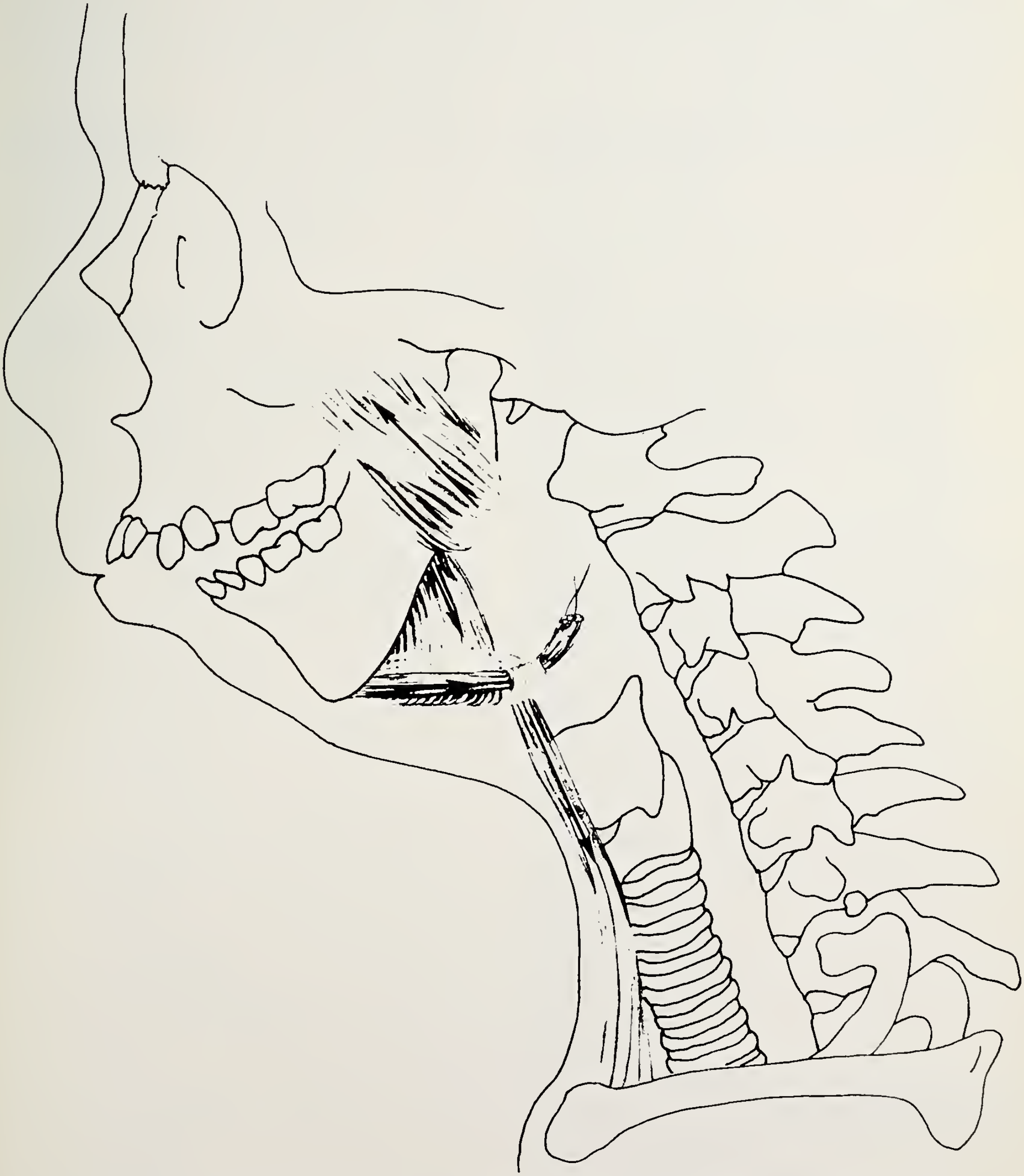

Figure 21. Illustrating the vectors of muscle tension producing antegonial notching in the presence of condylar arrest.

series of experiments of nature that affect the craniofacial complex, it is evident that they constitute fertile territory for the study of phenomena of oral sensation and perception. Such studies would not only provide fundamental knowldge but would inevitably contribute to improved diagnosis, prognosis and provide rational basis for therapy.

Finally, I am reminded of a recent article which appeared in *Science*, entitled "Coherent Social Groups and Scientific Change."[6] The authors observed that, "When challenged, some members of a scientific specialty become organized to work towards certain objectives voluntarily and self-consciously as a coherent and activist group. Such small, coherent activist groups subsequently had major impacts on their whole disciplines."

I believe this observation describes this conference and forecasts its impact on future directions in oral physiology.

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The presentations of the conference participants and their contributions to the discussions are in bold face type. Bibliographic references are in italics.

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